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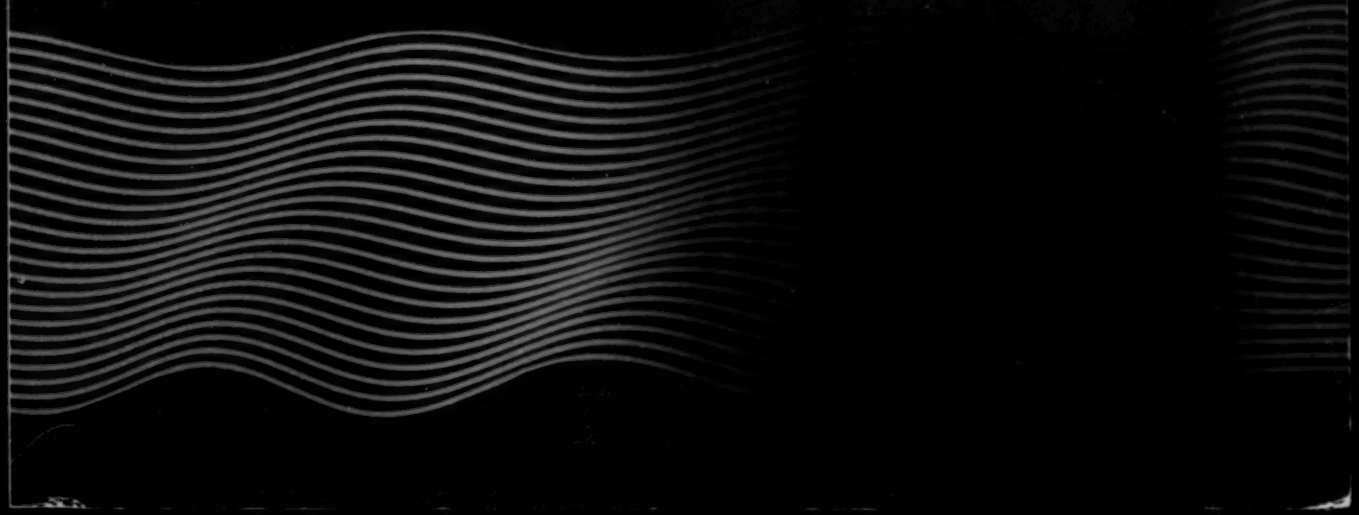

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Effects of surgically and gastrically implanted radio transmitters on swimming performance and predator avoidance of juvenile chinook salmon (*Oncorhynchus tshawytscha*)

Noah S. Adams, Dennis W. Rondorf, Scott D. Evans, Joseph E. Kelly, and Russell W. Perry

Abstract: Radiotelemetry data are often used to make inferences about an entire study population; therefore, the transmitter attachment method should be the one that least affects the study animal. Juvenile chinook salmon (*Oncorhynchus tshawytscha*) <120 mm in fork length (FL) with either gastrically or surgically implanted transmitters had significantly lower critical swimming speeds than control fish 1 and 19–23 days after tagging. For fish >120 mm FL, fish with gastric implants swam as well as controls 1 day but not 19–23 days after tagging. In contrast, fish with surgical implants swam as well as controls 19–23 days but not 1 day after tagging. During predation trials, fish with gastric or surgical implants were eaten by smallmouth bass (*Micropterus dolomieu*) in significantly greater numbers than controls. We do not recommend implanting transmitters (representing 4.6–10.4% of the fish's body weight) in fish <120 mm FL. Furthermore, surgical implants (representing 2.2–5.6% of the fish's body weight) may be the preferred method for biotelemetry studies of juvenile chinook salmon >120 mm FL.

Résumé : Comme le radio-pistage est souvent utilisé pour caractériser, par déduction, une population entière, le mode de fixation de l'émetteur doit nuire le moins possible au sujet radio-pisté. Nous avons constaté, chez des saumons quinnats (*Oncorhynchus tshawytscha*) juvéniles d'une longueur à la fourche de <120 mm qui portaient un émetteur ingéré ou chirurgicalement implanté, que la vitesse de nage en situation d'urgence était inférieure, dans une mesure significative, à celle des sujets témoins 1 jour, puis 19–23 jours après le marquage. Parmi les poissons de >120 mm, les sujets portant un émetteur ingéré nageaient aussi bien que les témoins 1 jour après le marquage, mais non 19–23 jours plus tard. Par contre, les sujets porteurs d'un émetteur chirurgicalement implanté nageaient aussi bien que les témoins 19–23 jours après le marquage, mais non le jour suivant le marquage. Durant les essais de prédation, l'achigan à petite bouche (*Micropterus dolomieui*) a capturé un nombre de poissons porteurs d'un émetteur, ingéré ou chirurgicalement implanté, nettement supérieur au nombre de poissons capturés parmi les témoins. Nous ne recommandons pas d'utiliser comme porteurs d'émetteur (lequel peut représenter de 4,6 à 10,4 % du poids du poisson) les poissons dont la longueur à la fourche est <120 mm. Par ailleurs, les implants chirurgicaux (qui peuvent représenter de 2,2 à 5,6 % du poids du poisson) sont peut-être préférables pour le radio-pistage du saumon quinnat juvénile d'une longueur à la fourche >120 mm.
[Traduit par la Rédaction]

Introduction

Following the construction of hydroelectric dams on the Snake River, salmon populations have declined (Nehlsen et al. 1991) and several have been classified as threatened or endangered (National Marine Fisheries Service 1992). To aid in the recovery of these populations, we are currently using biotelemetry to evaluate ways to improve the survival of juvenile salmonids as they pass hydroelectric dams on the Snake River, Washington. Although biotelemetry has allowed biologists and managers to gather data on fish movements that would otherwise be unattainable (Stuehrenberg et al. 1990; Wilson et al. 1991), use of this information to make inferences about an entire population assumes that a fish's movements are unaffected by the transmitter. To make this assumption, we must know whether the presence of the transmitter and the attachment procedure affect fish physiology and behavior.

Research conducted on juveniles and adults from a wide range of fish species provides some insight into the effects of various transmitter attachment methods on fish health and behavior. No consensus has been reached on the preferred method of attachment. Mellas and Haynes (1985) compared the effects of externally attached, surgically implanted, and gastrically implanted transmitters on swimming performance and behavior of adult rainbow trout (*Oncorhynchus mykiss*) and white perch (*Morone americana*). The authors concluded that a transmitter implanted in a fish's stomach may have the least affect on the study animal. However, other studies found that surgically implanted transmitters did not affect mortality, growth, or swimming behavior and may be preferred for use

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with adult rainbow trout (Lucas 1989) and juvenile Atlantic salmon (*Salmo salar*) (Moore et al. 1990). These studies indicate that the effects of a transmitter on the growth and activity of a test fish are partly determined by the method of attachment and appear to vary among species.

Regardless of the method of transmitter attachment, research conducted by Marty and Summerfelt (1986) and Greenstreet and Morgan (1989) indicates that adverse effects on fish physiology and behavior generally increase as the ratio of transmitter weight to fish weight increases. Winter (1983) recommended against using a transmitter that weighed more than 2% out of water of a fish's weight out of water. However, transmitters implanted in smaller juvenile salmonids may constitute more than 2.0% of the fish's body weight, which might affect survival, growth, or behavior. Hence, our objective was to determine the effects of surgically and gastrically implanted radio transmitters (representing 2.2–10.4% of the fish's body weight) on the swimming performance and predator avoidance of juvenile chinook salmon (*Oncorhynchus tshawytscha*).

Methods

Test fish and handling procedures

We used juvenile chinook salmon between 95 and 160 mm fork length (FL) from the Little White Salmon National Fish Hatchery (Washington) in all experiments. Fish were held in circular fiberglass tanks (1400 L, 1.5 m in diameter, 0.9 m deep) located outdoors under natural photoperiod and supplied with well water heated to 12°C. Water flow in each tank was maintained at the minimum amount needed to produce a circular current. Fish were fed Biomoist (Bioproducts, Warrendale, Oreg.) feed pellets to satiation three times a day at a rate of at least 3% of body weight per day. Fish were not fed 48 h before or 24 h after tagging.

Fish were randomly selected and subjected to one of three treatments: control (handled but not tagged), gastric implantation of transmitters (hereafter referred to as gastric fish), or surgical implantation of transmitters (hereafter referred to as surgery fish). Each dummy radio transmitter (manufactured by Advanced Telemetry Systems, Isanti, Minn.) consisted of real components encased in an inert resin (3M Scotchcast, electrical No. 5, Austin, Tex.) and a flexible antenna made of Teflon-coated stainless steel wire (0.5 mm in diameter, 31 cm in length). Dummy transmitters were representative of functional transmitters commonly used in the field. The body of the transmitters measured 5 × 14 mm and weighed 1.0 g in air (representing 2.2–10.4% of the body weight of the test fish) and 0.7 g in water (representing 1.5–7.3% of the body weight of the test fish).

Individual fish were anesthetized in a bath containing 70 mg tricaine methanesulfonate (MS 222)·L^{-1} buffered with sodium bicarbonate to a pH 7 and kept in the bath for 90 s after losing equilibrium (Summerfelt and Smith 1990). Fish were then freeze-branded with a unique mark, weighed to the nearest tenth of a gram, and measured to the nearest millimetre (fork length) before receiving one of the three treatments. Immediately after treatment, fish were placed in a freshwater recovery bucket (19 L, 28 cm in diameter, 35 cm deep) supplied with bottled oxygen at a rate of 50 mL·min^{-1}. As soon as fish regained equilibrium, they were transferred into one of four holding tanks. We supplied oxygen through an air stone into each holding tank at a rate of 200 mL·min^{-1} starting 20 min before handling and ending 20 min after the last fish had been returned to the tank. Holding fish in oxygenated water has been shown to reduce the stress associated with anesthesia (Hoar and Randall 1971; Sylvester 1975).

Implantation techniques

Procedures for gastric implantation of transmitters were similar to those used by Burger et al. (1985). Fish were positioned on their side on a Plexiglas surface lubricated with an artificial mucus restoring substance (Stress Coat; Aquarium Pharmaceuticals, Inc., Chalfont, Pa.). The transmitter was then gently pushed into the stomach using a Plexiglas tube (4 mm in diameter, 150 mm in length). The antenna was bent before implantation so that the portion protruding from the mouth pointed posteriorly.

Procedures for surgical implantation of transmitters were similar to those used by Moore et al. (1990), but were modified for use on fish between 95 and 160 mm FL. A soft foam pad with a groove cut in the center was soaked with Stress Coat and used to stabilize the fish's body during surgery. Fish were placed ventral side up on the pad and the gills were continuously flushed with anesthetic (MS 222, 20 mg·L^{-1}) fed through a tube placed in the fish's mouth. An in-line valve was used to control anesthetic flow and prevent contamination of the incision. The flow rate of the anesthetic varied with the size of the fish but averaged 250 mL·min^{-1}. About 1 min before completion of the surgical procedure, the flow of anesthetic solution was replaced with oxygenated freshwater to start the recovery process. Disinfection and sterilization of surgical equipment followed procedures described by Summerfelt and Smith (1990).

To implant the transmitter in the fish, a 10-mm-long incision was made 3 mm away from and parallel to the midventral line starting about 3 mm anterior to the pelvic girdle. The incision was only deep enough to penetrate the peritoneum (Summerfelt and Smith 1990). A 50 mg·kg body $weight^{-1}$ dosage of oxytetracycline (100 mg·mL^{-1}) was then pipetted into the incision to minimize infection (Hart and Summerfelt 1975; Summerfelt and Smith 1990). To provide an outlet in the body wall for the antenna, we used a shielded-needle technique similar to that described by Ross and Kleiner (1982). An intravenous catheter and needle (Abbocath-T No. G714, 16-gauge × 51 mm), with the hard plastic base of the catheter removed, was used to guide the antenna through the body wall of the fish. The catheter-covered needle was inserted through the incision to a point 5–10 mm posterior and slightly caudal to the origin of the pelvic fins. The point of the needle was exposed by pulling the catheter back onto the needle and pressure was then applied until both the needle and catheter pierced the skin of the fish. The needle was then pulled back out of the incision, leaving the catheter in position to guide the transmitter's antenna through the body wall of the fish.

The transmitter was implanted by first threading the antenna through the incision end of the catheter. Both the antenna and catheter were then gently pulled posteriorly while the transmitter was simultaneously inserted into the body cavity. The position of the transmitter inside the fish was adjusted by gently pulling on the antenna until the transmitter was horizontal and directly under the incision. The incision was closed with three simple, interrupted absorbable sutures (Ethicon coated vicryl braided, 5-0 taper RB-1 needle) evenly spaced across the incision. The antenna was attached to the side of the fish with a single suture in the caudal peduncle, about 5–6 mm posterior to the exit site. To prevent infection, a small amount of antibacterial ophthalmic ointment (Neobacimyx) was applied to all incisions (Summerfelt and Smith 1990). Previous research showed that the transmitter did not cause significant peritonitis or coelomitis 21 days after implantation (Adams et al. 1998).

Swimming performance

Swimming performance was evaluated using two modified Blaska respirometer–stamina chambers (Smith and Newcomb 1970). Each swimming chamber (17 cm inside diameter and 90 cm in length) was longitudinally divided into four compartments, thus allowing eight fish (one fish in each compartment of the two swimming chambers) to be tested simultaneously (Fig. 1). Black plastic was draped over the upper 60 cm of the swimming chamber and a bright light was placed at the downstream end of the chamber to encourage fish to swim and provide a visual cue for orientation of swimming fish (Griffiths and Alderdice 1972). We further encouraged fish to swim by operating an

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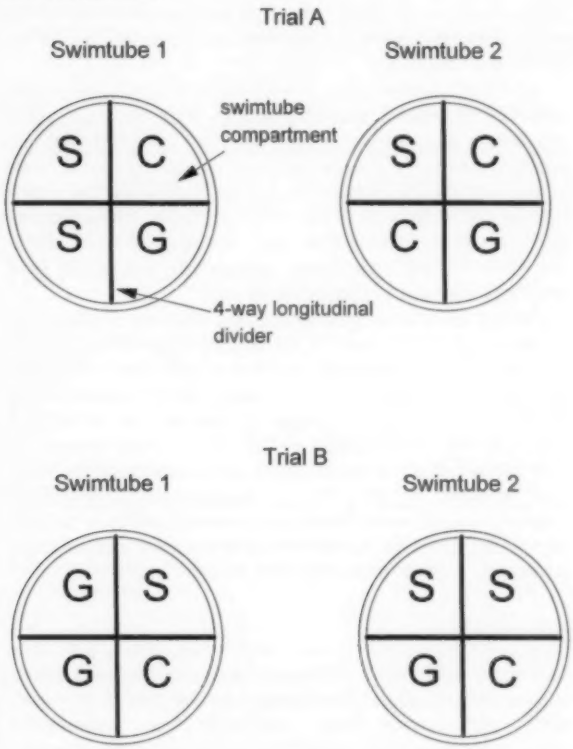
Fig. 1. Cross-sectional view of the swimming chambers used to test the swimming performance of juvenile chinook salmon. Each swimming chamber was longitudinally divided into four compartments, thus allowing eight fish (one fish in each compartment of the two swimming chambers) to be tested simultaneously. Fish were randomly selected and subjected to one of three treatments (C, control; G, gastric; S, surgery). To account for potential compartment effects, fish from each treatment type were systematically assigned to a swimming compartment. By controlling in which compartment fish were tested, we ensured that an equal number of fish from each treatment type were tested in each compartment.



electrified screen (15–20 V AC) located at the downstream end of the swimming chamber (Brett 1964; Bams 1967; Beamish 1978; Mellas and Haynes 1985; Williams and Brett 1987). During the tests, well water heated to 13°C was circulated through the swimming chamber by an electric motor driving a propeller. Water velocity was varied by altering the current to the electric motor. To calibrate the swimming chambers, we used linear regression to assess the relationship between water velocity (measured in centimetres per second using a Swoffer velocity meter probe placed in the center of the swimming chamber) and motor speed (Mesa and Olson 1993).

To determine the effects of transmitter implantation on swimming performance, fish were tested 1 day (1 day = 24 h) and 19–23 days (hereafter referred to as 21 days) after treatments were applied. Long-term effects were examined at 21 days because the functional life of the transmitter we currently use to study juvenile fish movements does not exceed 21 days. However, transmitters can be configured to function for 3 months or more by delaying activation of the transmitter and decreasing the rate at which it emits a signal. Because only 16 fish could be tested per day (eight in the morning and eight in the afternoon; Fig. 1, trials A and B), fish were treated and tested in groups of 16 fish each. For both the 1- and 21-day tests, eight groups totaling 128 fish (44 control fish, 42 gastric fish, and 42 surgery fish) were treated and then tested. To ensure that all four swimming chambers would be occupied during each trial and to compensate for potential mortality or expulsion of transmitters during the holding period, extra control fish were treated for the 21-day experiment.

To start a swimming performance trial, fish were randomly selected from the holding tanks and measured to the nearest millimetre (fork length). One fish was then placed in each of the four swimming compartments and the average fork length of all four fish was used to calculate water velocities for acclimation and testing. To account for potential compartment effects, fish from each treatment type were systematically assigned to a swimming compartment (Fig. 1). By controlling which compartment fish were tested in, we could ensure that an equal number of fish from each treatment type were tested in each compartment. Fish were allowed to acclimate to the swimming chambers for 1 h at 0.5 body length (BL)·s^{-1}. After acclimation, water velocity was increased by 0.5 BL·s^{-1} every 15 min until fatigue occurred (Beamish 1978; Moser et al. 1990). A fish was considered fatigued when it became impinged on and would not leave the electrified screen at the back of the chamber despite three 1-s shocks (15–20 V AC) in a 10-s period. Critical swimming speed (U_{crit}) was calculated following procedures described by Beamish (1978). Although the presence of a fish in the swimming chamber can cause solid blocking effects (Bell and Terhune 1970), we did not correct for these effects because all of our fish had cross-sectional areas <10% of the cross-sectional area of the swimming chamber (Brett 1964). Although edge effects (Beamish 1978) likely existed within the swimming compartments, they were not considered when determining values of U_{crit} because we were only interested in relative differences between treatment types and the experimental design accounted for potential compartment effects.

Because fish size can affect U_{crit} (Brett 1964), linear regressions of U_{crit} on fork length were performed for each treatment group at 1 and 21 days. The data did not meet the assumption of homogenous slopes for analysis of covariance (ANCOVA). Therefore, data were grouped into two size categories (<120 mm FL and ≥120 mm FL) and analyzed by analysis of variance (ANOVA) and then by multiple comparisons of means using the Tukey–Kramer procedure (Sokal and Rohlf 1981). We selected these size categories based on scatter plots of U_{crit} versus fork length and observations we made during previous field studies. Scatterplots indicated that transmitter implantation had a greater effect on the swimming performance of fish <120 mm FL, and radio-tagged fish of this size displayed abnormal swimming behavior and appeared to have difficulty compensating for the weight of the transmitter during field studies.

Predator avoidance

Predation trials were conducted between 15 September 1995 and 17 October 1995 in two 9500-L circular fiberglass tanks (3.7 m in diameter, 0.9 m deep). A cobble substrate and seven lengths of polyvinyl chloride pipe (15 cm in diameter, 35 cm in length) in each of the tanks provided cover for the test fish. Tanks were located indoors and supplied with well water, heated to 15°C, at a rate of 8 L·min^{-1}. Four 40-W incandescent bulbs evenly spaced above each tank and controlled by timers simulated a natural photoperiod. Platforms suspended 2 m above the tanks allowed us to observe predator–prey interactions with minimal disturbance to test fish. Observations were made during 15-min blocks immediately after prey were introduced and at 2-h intervals thereafter. No observations were made at night.

Fourteen smallmouth bass (*Micropterus dolomieu*) (average FL = 30.6 cm), collected from the Columbia River by electroshocking, were used as predators. Seven smallmouth bass were placed in each of the two test tanks and allowed to acclimate for 30 days before the predation trials. During acclimation, smallmouth bass were fed a maintenance diet of live juvenile chinook salmon, but were not fed 48 h before predation trials.

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To start a trial, 12 control and 12 tagged fish (gastric or surgery fish) were simultaneously placed into each of the two predation tanks 1 day after treatment. Fish used in the predation trials were not the same fish used in swimming performance tests. Predation was allowed to continue until 30–50% of the prey were consumed by smallmouth bass. Four trials using gastric and control fish and four trials using surgery and control fish were conducted in each of the two predation tanks. After each trail, all remaining prey were removed from the tanks. At the end of the study, necropsies were conducted to determine if transmitters implanted in the prey had become lodged in the digestive systems of the smallmouth bass.

All data were subjected to a heterogeneity chi-square analysis to determine if the predation trials were homogenous (Sokal and Rohlf 1981). Data were pooled for trials using gastric and control fish and surgery and control fish. Chi-square goodness-of-fit tests were then conducted to determine if predation was random (50:50, gastric: control fish and surgery:control fish consumed).

Results

Swimming performance

During the 21-day holding period, one gastric fish died on day 14 and one surgery fish was killed on day 11 to examine fungus growing along the incision wound (later identified as *Saprolegnia* sp.). In addition, three surgery fish tangled their antennas around the center standpipe of the tank and pulled the transmitter out through the opening for the antenna. During the 21-day swimming performance trials, three gastric fish and three surgery fish had their antennas tangle in the center partition of the swimming chamber. Similarly, during the 1-day swimming performance tests, two gastric fish and two surgery fish had their antennas tangle in the center partition of the swimming chamber. Although none of the fish that tangled their antennas died during the experiment, they were excluded from the swimming performance analysis. During the 21-day swimming trials, extra control fish were tested so that all compartments of the swimming chamber were occupied.

Linear regressions of U_{crit} on fork length for 1- and 21-day tests revealed that smaller control fish had higher U_{crit} values than larger control fish; slope of the line was significantly different from zero ($P = 0.0001$). This relationship was not evident for gastric or surgery fish; slope of the line was not significantly different from zero ($P > 0.05$). These results supported our observation that transmitter implantation had a greater effect on the swimming performance of small fish (<120 mm FL). Before the data were pooled to make comparisons between size categories, further analysis revealed no significant time-of-day effect, no significant group effect, no significant swimtube effect, and a significant interaction between treatment and tube (ANOVA, $P < 0.05$). There was not a true interaction between treatment and tube because, by chance, a disproportional number of the small radio-tagged fish were tested in one of the two swimtubes. When length was included in the model as a covariate, there was no significant interaction between treatment and tube.

After the data were pooled and grouped into size categories (<120 mm FL and ≥120 mm FL), we found a significant treatment effect at 1 and 21 days after tagging (ANOVA, $P < 0.05$). For fish <120 mm FL, multiple comparisons revealed that gastric and surgery fish had significantly lower U_{crit} values than controls 1 and 21 days after tagging. For fish >120 mm FL, gastric fish swam as well as controls 1 day but not 21 days after tagging. In contrast, surgery fish swam as well as controls 21 days but not 1 day after tagging (Table 1).

Predator avoidance

Tests for heterogeneity among the predation trials were not significant ($0.01 < P < 0.05$); therefore, the data were pooled. Over all predation trials, the ratio of gastric to control fish and surgery to control fish eaten was identical (72%:28%, tagged:control fish consumed; Tables 2 and 3). Upon release, juvenile chinook salmon dispersed throughout the tank and a few fish were consumed. Shortly after release, when the number of predator attacks increased, the prey formed a tight school near the top of the water column. Prey fish remained in a school and swam in a circular pattern around the tank for the duration of the trial.

Although smallmouth bass were inactive during most of our observations, some successful attacks were recorded. After consuming one or more tagged fish, we observed antennas protruding from the smallmouth bass' mouths. However, none of the smallmouth bass had antennas protruding from their mouths before the start of any of the predation trials. Necropsies revealed that transmitters were not retained in the digestive system of the smallmouth bass, where they might have interfered with normal digestion of prey.

During the first six predation trials, when the average size of the prey was <120 mm FL, some of the tagged fish appeared to have difficulty swimming with the school. None of the fish appeared to have difficulty swimming with the school during the last six predation trials, when the average size of the prey was >120 mm FL (Tables 2 and 3). As the mean size of the prey increased, the duration of the individual predation trials also increased. For the first six predation trials, the desired level of predation (30–50% of the prey consumed) was reached after 1 day. In contrast, only two of the last six trials reached the desired level of predation after 3 days.

Discussion

Although small juvenile chinook salmon survived with gastrically or surgically implanted transmitters, results of the swimming performance tests indicated that transmitters representing 4.6–10.4% of the fish's body weight were unsuitable for use in fish <120 mm FL. When the same transmitters (representing 2.2–5.6% of the fish's body weight) were implanted in fish >120 mm FL, the poor swimming performance of gastric fish by day 21 suggested that surgical implantation may be the preferred method for use in long-term biotelemetry studies. However, data gathered on fish movements 1 day after release may not be useful because swimming performance was impaired 1 day after surgery.

Swimming tests have been used to examine the effects of transmitter implantation on fish performance, and other researchers have reported a correlation between swimming performance and fish size. For example, McCleave and Stred (1975) noted that 1 day after radio tagging, larger juvenile Atlantic salmon with gastric implants swam better than smaller fish. Our results were similar to these except for differences in U_{crit} values between the species (2.3–5.3 $BL \cdot s^{-1}$ for juvenile chinook salmon; 2.2–2.5 $BL \cdot s^{-1}$ for juvenile Atlantic salmon). Because a fish's relative ability to perform decreases as size increases (Brett 1964), differences in U_{crit} values may be

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Table 1. Mean relative critical swimming speeds (U_{crit}, BL·s^{-1}) ± 95% confidence interval and range (in parentheses) 1 and 21 days after treatment for juvenile fall chinook salmon <120 mm FL and ≥120 mm FL.

Treatment	*N*	<120 mm FL	*N*	≥120 mm FL
		1-day relative U_{crit}		
Control	17	4.66±0.86 (3.75–5.29) *a*	27	3.90±0.75 (3.05–4.64) *a*
Gastric	14	3.67±1.17 (2.47–4.91) *b*	26	3.69±0.63 (2.85–4.26) *ab*
Surgery	8	3.74±0.63 (3.22–4.26) *b*	32	3.45±0.92 (2.20–4.49) *b*
		21-day relative U_{crit}		
Control	12	4.51±0.63 (3.98–5.02) *a*	36	3.97±0.92 (2.75–4.81) *a*
Gastric	18	3.58±0.79 (2.63–4.11) *b*	20	3.55±0.63 (2.64–4.03) *b*
Surgery	19	3.91±1.11 (2.70–5.03) *b*	16	3.82±0.79 (3.18–4.73) *ab*

Note: Means within a column followed by the same letter are not significantly different ($P > 0.05$).

Table 2. Predation on juvenile chinook salmon 1 day after gastric implantation of radio transmitters.

Trial	Mean FL (mm)	Number eaten		% total eaten	Statistics	
		Gastric	Control		df	χ^2
1 (24)	113	7	4	46	1	0.818
2 (24)	110	8	4	50	1	1.333
3 (24)	114	8	2	42	1	3.600
4 (24)	110	6	0	25	1	6.000*
5 (36)	117	7	2	38	1	2.778
6 (72)	128	4	5	38	1	0.111
7 (72)	124	5	1	25	1	2.667
8 (72)	125	4	1	21	1	1.800
Total		49	19		8	19.107*
Pooled					1	13.235***
Heterogeneity					7	5.872

Note: For all trials, $N = 12$ for gastric and control fish. The duration (h) of each trial is given in parentheses. Asterisks denote predation rates that differ significantly (*$P < 0.05$ or ***$P < 0.001$) from expected (1:1, gastric:control fish).

Table 3. Predation on juvenile chinook salmon 1 day after surgical implantation of radio transmitters.

Trial	Mean FL (mm)	Number eaten		% total eaten	Statistics	
		Surgery	Control		df	χ^2
1 (24)	119	6	3	38	1	1.000
2 (24)	112	7	2	38	1	2.778
3 (24)	113	7	1	32	1	4.500*
4 (24)	115	9	2	46	1	4.455*
5 (36)	118	6	2	33	1	2.000
6 (72)	126	5	1	25	1	2.667
7 (72)	124	3	6	38	1	1.000
8 (72)	129	3	1	17	1	1.000
Total		46	18		8	19.399*
Pooled					1	12.250***
Heterogeneity					7	7.149

Note: For all trials, $N = 12$ for surgery and control fish. The duration (h) of each trial is given in parentheses. Asterisks denote predation rates that differ significantly (*$P < 0.05$ or ***$P < 0.001$) from expected (1:1, surgery:control fish).

attributed to differences in the size of the test fish. Fish used in our study were smaller (mean FL = 125 mm) than those used by McCleave and Stred (1975) (mean total length = 218 mm).

For fish >120 mm, gastric fish swam as well as controls 1 day but not 21 days after tagging. Others have shown that gastrically implanted transmitters did not affect swimming performance up to 7 days after tagging (McCleave and Stred 1975; Mellas and Haynes 1985; Moser et al. 1990). In our study, the poor swimming performance of gastric fish by day 21 may be attributed to the effect of the transmitter on food consumption. If gastric fish had difficulty eating because of the presence of the transmitter, less energy would be available for swimming. Previous research conducted in our laboratory showed that transmitters placed in the stomach of juvenile chinook salmon <140 mm FL impaired the normal ingestion of food and resulted in growth rates that were significantly lower than those of controls by days 21 and 54 of the study (Adams et al. 1998).

Surgically implanted transmitters did not cause significant long-term effects on the swimming performance of fish >120 mm FL. These results are similar to those reported by other researchers. The swimming capacity of juvenile Atlantic salmon with surgical implants was not affected during a 7- to 14-day period (Moore et al. 1990). Likewise, adult rainbow trout with surgical implants performed as well as controls during swimming tests conducted 7 days after tagging (Mellas and Haynes 1985). Our results showed, however, that surgically implanted transmitters affected swimming performance 1 day after tagging. This short-term effect may be the result of postoperative stress. Surgical implantation may be more stressful on the test fish than gastric implantation because it requires more handling, anesthesia, and recovery time. Gastric implantation took about 30 s whereas surgical implantation required about 3.5 min. Chronic and multiple acute forms of stress, like surgical procedures, have been shown to adversely affect fish physiology (Pickering et al. 1982) and behavior (Sigismondi and Weber 1988).

Previous research has shown a relationship between swimming performance and vulnerability to predation (Bams 1967). This correlation may partially explain why the ratio of gastric to control fish and surgery to control fish eaten was identical (72%:28%, tagged:control fish consumed). The mean size of the prey was <120 mm FL in five of eight trials using gastric and control fish and in five of eight trials using surgery and control fish. For fish <120 mm FL, gastric and surgery fish had significantly lower U_{crit} values than controls 1 day after tagging. A correlation between swimming performance and

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vulnerability to predation may also have accounted for the increased duration of the predation trials. The mean size of the prey ranged from 124 to 129 mm FL in the last three trials using gastric and control fish and the last three trials using surgery and control fish. Fish >120 mm FL had U_{crit} values that were more comparable with those of the controls, perhaps making it more difficult for the smallmouth bass to capture prey.

Although predation trials lasted longer as the mean size of the prey increased, gastric and surgery fish were still eaten in greater numbers than control fish. This suggested that factors other than U_{crit}, including failure to detect predators, decreased fast-start performance, inability to shoal effectively, and increased conspicuousness (Mesa et al. 1994), could have influenced the vulnerability of radio-tagged prey to predation. It is likely that several of these mechanisms, and perhaps others not listed, worked in concert to elicit differential predation. For example, swimming performance was affected by the transmitter, and the transmitter antenna could have made it easier for predators to target and capture tagged fish.

We do not recommend surgically or gastrically implanting transmitters (representing between 4.6 and 10.4% of the fish's body weight) in fish <120 mm FL. In larger fish, surgical implants (representing 2.2–5.6% of the fish's body weight) did not cause significant long-term effects on swimming performance, and even though swimming performance was impaired 1 day after tagging, surgial implants did not cause higher predation rates than gastric implants. Because movements of fish with surgically implanted transmitters should be more similar to those of untagged fish, we recommend this method for biotelemetry studies of juvenile chinook salmon >120 mm FL. Furthermore, surgically implanted transmitters may allow for better long-term survival of the test fish and appear preferable for studying endangered salmon populations.

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Nucleic acids and enzymes in Atlantic cod (*Gadus morhua*) differing in condition and growth rate trajectories

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Abstract: Atlantic cod (*Gadus morhua*) were exposed to one of four feeding regimes, 16 weeks of food deprivation (U) or satiation feeding (F) or two 8-week periods of food deprivation followed by satiation feeding (UF) or vice versa (FU), to determine whether relationships between nucleic acids or enzymes and growth rates result from a general enhancement of individual condition or are a direct result of enhanced growth rates. Final condition factor (K) differed between treatments, but did not differ between the mixed treatments after either 8 weeks of negative growth (FU) or 8 weeks of positive growth (UF). Intestinal cytochrome *c* oxidase activity matched the expected short-term growth rates, not only in fed and unfed fish but also in cod exposed to the mixed treatments (FU and UF). White muscle lactate dehydrogenase activity reflected growth rates, but initial levels were not reached within 8 weeks in FU cod. The liver glutamate pyruvate transaminase : DNA ratio reflected differences in K, but not differences in recent growth rates. Myofibrillar proteins decreased in unfed cod, while sarcoplasmic proteins followed changes in K more closely. The RNA:DNA ratio in white muscle did not reflect changes in K or changes in growth rates.

Résumé : Des morues franches (*Gadus morhua*) ont été soumises à quatre régimes alimentaires, soit 16 semaines de jeûne (U) ou d'alimentation à satiété (F) ou deux régimes mixtes, 8 semaines de jeûne suivies de 8 semaines à satiété (UF) ou vice versa (FU), pour déterminer si les corrélations observées entre les acides nucléiques ou les enzymes et le taux de croissance sont le fait d'une amélioration de la condition ou la résultante d'un changement du taux de croissance. L'indice de condition final (K) variait entre les traitements, mais non entre les traitements mixtes, suite à 8 semaines de décroissance (FU) ou 8 semaines de croissance (UF). L'activité de la cytochrome *c* oxydase dans l'intestin reflétait le taux récent de croissance, non seulement chez les poissons à jeun ou nourris, mais aussi chez les poissons des traitements mixtes (FU et UF). L'activité de la lactate déshydrogénase du muscle blanc reflétait le taux de croissance, mais 8 semaines de jeûne n'ont pas suffi pour revenir au niveau initial chez les morues du groupe FU. Le ratio glutamate pyruvate transaminase : ADN du foie variait plutôt en fonction de K. Les protéines myofibrillaires n'ont diminué que chez les poissons à jeun (U) alors que les protéines sarcoplasmiques s'ajustaient à K. Le ratio ARN:ADN du muscle blanc n'a varié ni en fonction de K ni en fonction du taux de croissance.

Introduction

Traditional methods used for measuring growth rates in fisheries research have yielded little insight into growth–environment relationships. Growth rates calculated from changes in size at age using bony structures as age markers or using methods based on mark–recapture or length–frequency data are generally not adequate for the study of short-term changes in growth rates. Nutritional status of individuals assessed by proximate composition (Dutil et al. 1995; Lambert and Dutil 1997*a*) may be adequate (Holdway and Beamish 1984). Growth is, however, a complex process influenced by many factors in the environment, some of them changing over days and others over longer periods. Condition indices and proximate composition are static indicators of past growth performance. They cannot be used to assess recent growth history unless measured periodically and in a suitable time scale. Intermediate levels of hepatic lipids could, for example, result from intensive feeding of a starved animal or starvation of a well-fed animal.

There are a variety of other methods to assess growth rates that do not require measuring changes in size or tissue composition. Protein synthesis can be estimated by monitoring the rate of incorporation of radioactive amino acids (Houlihan et al. 1988). Nucleic acids reflect nutritional status and growth rate in several fish species (Bulow 1970; Buckley 1979, 1984; Mathers et al. 1992; Foster et al. 1993). Finally, mitochondrial enzymes have been investigated under the general hypothesis that their activity would be adjusted to meet the metabolic demand for growth. Positive correlations between mitochondrial enzymes in white muscle and growth rates have been found in pollock (*Pollachius virens*) (Mathers et al. 1992). Enzymes of glycolytic (lactate dehydrogenase (LDH), in

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muscle), oxidative (cytochrome *c* oxidase (CCO), in intestine), and amino acid (glutamate pyruvate transaminase (GPT), in liver) metabolism correlate well with growth rates in Atlantic cod (*Gadus morhua*), but stronger correlations were observed for glycolytic enzymes than for mitochondrial enzymes in white muscle (Pelletier et al. 1993*a*, 1993*b*, 1994). These methods have a common advantage, i.e., they provide an individual and instantaneous measure of growth rates. Depending on their degree of lability, they may provide valuable information on the relationship between fish performance and changes in environmental conditions or in food availability.

The protocol used to validate nucleic acids or enzymes as indicators of growth rates generally consists of setting up experiments in which food availability is controlled and in which changes in size are measured and expressed as average growth rates for that period. Given appropriate time scales of observations, such protocols would favor correlations between the indicator and growth rates, and these correlations have been used to assess the growth rates of fish in the wild (Mathers et al. 1992; Guderley et al. 1996). There is, however, a potential problem with such procedures: not only does the size increase, but the condition of the fish also increases as energy reserves are being built up. Thus the general condition of fish changes in parallel with changes in mass. Based on an examination of correlations between the indicator and growth rates, one cannot conclude whether changes in the indicator are a direct reflection of enhanced growth rates as such or whether they reflect a general enhancement of individual condition. This study examines whether adjustments in the concentrations of muscle nucleic acids and in the activity of several enzymes in muscle, liver, and intestine are correlated with recent growth rates of fish that have similar levels of energy reserves. This was achieved by manipulating the feeding regime so that fish would reach similar condition factor values, a measure of energy reserves in cod (Lambert and Dutil 1997*a*), at the end of the experiment following either a period of negative (FU) or positive (UF) growth rates. If nucleic acids and enzymes differ between the mixed treatments (FU and UF), i.e., in the absence of any difference in energetic condition, then we may conclude that nucleic acids and enzymes are adjusted to growth rates. Starved and fed fish for which condition factor was not controlled were used as references. Potential size effects on the response of nucleic acids and enzyme activities to growth and changes in condition were examined by using two size-classes of fish. This was important considering the allometric effects that govern the levels of many biochemical parameters (Somero and Childress 1980; Goolish 1991; Pelletier et al. 1993*a*, 1993*b*, 1994).

Materials and methods

Fish holding and experimental conditions

Wild cod were trawled from the St. Lawrence estuary in June 1994 and put into large circular tanks at Institut Maurice-Lamontagne, Mont-Joli, Que. The fish recovered for 4 weeks in running seawater (30‰, 5–9°C). Few mortalities occurred during that period. Cod were treated against external parasites with formalin (0.02%). They were fed capelin twice a week. The tank room was lit with low-intensity fluorescent lights controlled by automatic timers to follow the natural photoperiod at our latitude.

Fish were anaesthetized in a solution of metomidate hydrochloride (4 mg·L^{-1}), measured (±1 mm), weighed (±1 g), and received a Visual Implant Tag (Northwest Marine Technology, Shaw Island, Washington) in the first dorsal fin. The cod were then distributed among eight 1.5-m^3 tanks (24 fishes·tank^{-1}). Five tanks received cod larger than 40 cm (range 402–543 mm and 403–1357 g) and three tanks received cod smaller than 40 cm (range 295–400 mm and 196–500 g). Within size-classes, there were no significant differences in mean length (mean ± SD: 452 ± 33 and 364 ± 25 mm) or mass (mean ± SD: 687 ± 190 and 352 ± 78 g) of the cod in the different tanks ($P > 0.05$).

Larger fish were not fed (two tanks, U) or were fed frozen capelin to satiation (one tank, F) three times a week. Fed cod received all they could eat within 1 h. Larger fish in two additional tanks were either fed for 8 weeks and not fed for the remaining 8 weeks (FU) or vice versa (UF). Smaller fish were not fed (two tanks, U) or were fed to satiation (one tank, F). Smaller fish were not exposed to the mixed feeding regimes (FU and UF). The experiment lasted 16 weeks except for starved cod which were sampled when 50% of the cod had died to ensure that minimal energy reserves had been reached. The results reported here were obtained from survivors exclusively. Twenty fish were also sampled (smaller fish: 382 ± 13 mm, 384 ± 59 g, $n = 9$; larger fish: 448 ± 26 mm, 669 ± 169 g, $n = 11$) from the stock tanks (7.5°C) at the start of the experiment to determine reference levels. The experiment was conducted at 10.0 ± 0.5°C. Holding conditions and experimental protocols followed the principles outlined by the Canadian Council on Animal Care. The length of the feeding (F) and starvation (U) periods was determined so as to cover the whole range of condition factor values observed for the species in the wild, including very low condition factor values recently observed in cod in the northern Gulf of St. Lawrence (Lambert and Dutil 1997*b*). This was required to assess samples collected in the field (J.-D. Dutil and Y. Lambert, unpublished data).

Tissue sampling and assays

The fish were stunned by a blow to the head, measured (fork length, ±1 mm), and weighed (±1 g). Liver and gonads were dissected out and weighed (±0.1 g). White muscle was sampled from below the first dorsal fin and above the lateral line. The entire intestine from the distal end of the stomach to the vent was dissected out and any remaining feces were removed with saline solution. Liver and muscle samples and the whole intestine were frozen in liquid nitrogen and subsequently stored in a freezer (–80°C). Liver somatic index was calculated as liver mass divided by final somatic mass multiplied by 100. Instantaneous rate of growth was calculated as the natural logarithm of final mass minus the natural logarithm of initial mass multiplied by 100 and divided by the time interval in days. Initial condition factor was calculated as initial mass and final condition factor as final somatic mass (grams) multiplied by 100 and divided by fork length (centimetres) cubed. Gonadal somatic index averaged 0.9% in controls at the start and ranged between 0.5 and 1.5% among treatments at the end of the experiment and hence did not preclude comparing condition factor values based on somatic or total mass.

Muscle and liver samples were subsampled. The whole intestine was cut transversally into small pieces and a subsample made of several pieces was used to prepare tissue extracts. Tissue extracts were prepared by homogenizing 200–350 mg in 10 volumes of 50 mM imidazole–HCl, 2 mM $MgCl_2$, 5 mM EDTA, 1 mM reduced glutathione, 0.1% Triton X-100, pH 7.5, using an IKA homogenizer model T25 (Inter Science Inc., Markham, Ont.) for two 20-s periods. Between periods of homogenization, samples were put on ice for 1 min. For white muscle, crude extracts were used to avoid the loss of enzyme activity through binding to subcellular particles. Liver and intestine samples were homogenized in the presence of 0.1 mM phenylmethylsulphonyl fluoride, a general protease inhibitor (Moon and Mommsen 1987). The liver extract was centrifuged at 10 000 × *g* for 30 min to clarify the supernatant. To avoid precipitation of mitochondrial fractions, intestinal extracts were centrifuged at 1000 × *g* for 15 min (Pelletier et al. 1994).

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Maximal enzyme activities were measured at 10°C using a UV/Vis spectrophotometer (Beckman DU640) equipped with a thermostatted cell holder and circulating refrigerated water bath. Assay conditions followed Guderley et al. (1996). Conditions for the enzyme assays were as follows (all concentrations in millimoles per litre). LDH (EC 1.1.1.27): potassium phosphate (100), NADH (0.15), pyruvate (0.8), pH 7.0; CCO (EC 1.9.3.1): potassium phosphate (100), reduced cytochrome *c* (0.07), pH 7.0; reactions were run against a control and reduced cytochrome was prepared as described in Guderley et al. (1996); GPT (EC 2.6.1.2): potassium phosphate (50), alanine (100), NADH (0.2), pyridoxal phosphate (0.025), excess LDH, α-ketoglutarate (10), pH 7.4. Results are expressed as micromoles of substrate converted to product per minute (international units).

Protein fractions were assessed following the method outlined in Guderley et al. (1996). Centrifugation speed and time were modified to 20 000 × *g* for 3 min. Buffering capacity was estimated using the methods described in Guderley et al. (1996).

Nucleic acid assays

The nucleic acids assay method was adapted from Mayrand et al. (1998), who modified the Berdalet and Dortch's (1991) method to make it work with animal tissues, taking into account the recommendations made by Karsten and Wollenberger (1972, 1977) and Grémare and Vétion (1994). Essentially, this method quantifies total nucleic acids by their interaction with thiazole orange (TO), which binds to the polymerized forms. DNA is quantified after enzymatically eliminating the RNA in the sample with RNase. Heparin facilitates dissociation of the nucleic acids from the chromatin (Karsten and Wollenberger 1977). Background fluorescence measurements in the absence of TO are necessary, given the contributions of endogenous metabolites to the fluorescence.

TO (Molecular Probes Inc., Eugene, Oreg.) was prepared in absolute ethanol and was kept in the dark at room temperature. Calf thymus DNA, Baker's yeast RNA, RNase, and ammonium heparin were prepared in 0.2 M Tris–HCl, 0.1 M NaCl, 1 mM $CaCl_2$, pH 7.5. Solutions were kept at 4°C, except for the RNA and DNA stock solutions which were kept at –80°C.

Muscle extracts were prepared by homogenizing on ice 250–300 mg (fresh mass) of tissue in 10 mL of ice-cold Tris–Ca buffer (see above) using a homogenizer (IKA model T25) for two 20-s periods. Between periods of homogenization, samples were kept on ice for a few minutes. The extracts were then centrifuged at 3000 × *g* for 10 min at 4°C. Supernatants were kept on ice for the DNA and RNA assays. Liver extracts were prepared by homogenizing 150 mg of tissue in 10 mL of the above buffer containing 0.2% Triton X-100 (Fisher Scientific Co., Montreal, Que.). The homogenates were first centrifuged at 4500 × *g* for 15 min at 4°C. A 1-mL aliquot of supernatant was then diluted in 3 mL of Tris–Ca buffer (without Triton) and centrifuged again at 4500 × *g* for 10 min at 4°C. Supernatants were kept on ice for the DNA and RNA assays. Because the second assays required that we remove RNA selectively using RNase, we could not use SDS to improve recovery of nucleic acids, as SDS inhibits enzyme activity (Clarke et al. 1989). Preliminary tests showed that 0.0083% Triton X-100 had no effect on fluorescence or RNase activity while causing less bubbling than higher concentrations. Readings were carried out at 511 nm excitation wavelength and 533 nm emission wavelength on a Perkin-Elmer LS50 spectrofluorimeter.

Statistical methods

Each variate was checked with Shapiro–Wilk's test for normal distribution and Levene's test for homogeneity of variances. When one or both criteria were not met, data were log transformed. Size-class effects were tested with a two-factor ANOVA (size-class and feeding regime). Mixed regimes were excluded from this analysis because they used only large cod. When size-class effects were not significant, mean values were calculated for the two size-classes combined and were reported as such in the tables. Feeding regime effects (including the mixed treatments) were tested with a one-factor ANOVA on the pooled results from the two size-classes combined. Tests conducted on large fish exclusively led to the same conclusions as those on size-classes combined. Pairwise comparisons were conducted on least-square means. When underlying assumptions (normal distribution and homogeneity of variances) could not be met, data were ranked and tests made on rank scores. Differences were considered significant at the 1% level. The analyses were conducted using SAS software (SAS Institute Inc., Cary, N.C.).

Results

Final condition factor and growth rates averaged over the 16-week duration of the experiment were correlated (Fig. 1). Lowest condition factors occurred in cod with negative growth rates and highest condition factors occurred in cod that also had the highest growth rates during the experiment.

Final condition factor differed among treatments (Table 1). Condition factor averaged 0.74 ± 0.08 (n = 158) at the start of the experiment and there were no differences between size-classes and among treatments. Sixteen weeks later, F cod had a significantly higher condition factor and liver somatic index than U cod. The condition factor of cod in the mixed treatments (FU and UF) was intermediate (0.84 ± 0.10, n = 46) and did not differ between treatments, but recently fed cod (UF) had a higher liver somatic index (6.18) than recently starved cod (FU, 3.84). Differences in the liver somatic index between small and large cod were significant ($P < 0.01$), but differences in condition factor were not ($P > 0.01$).

Growth rates over the whole 16-week period followed a similar pattern as for condition factor (Table 1). Fish in the mixed treatments had similar growth rates, slower growth rates than F cod, and faster growth rates than U cod. Although FU and UF cod did not differ in condition factor or averaged overall growth rates, FU and UF cod reached the same condition factor following two different trajectories in the second half of the experiment, an 8-week period of starvation for FU cod and an 8-week period of positive growth for UF cod.

Enzyme activity on a wet mass basis differed among treatments. Starved cod had significantly lower muscle LDH and intestinal CCO activities than cod at the start of the experiment (Table 2). Fed cod on the contrary had higher LDH and CCO activities than cod at the start of the experiment. UF cod had similar LDH and CCO activities as fed cod. FU cod, on the other hand, had significantly lower activities than cod in the F or UF treatments. LDH activity in FU cod was intermediate between F cod and cod at the start of the experiment, but CCO activity in FU cod was similar to that measured at the start of the experiment. Fish size had no significant effect on the activity of either enzyme ($P > 0.01$).

Liver GPT (expressed as units per gram) responded differently to our treatments than muscle LDH and intestinal CCO because changes in GPT activity coincided with changes in liver somatic index (Table 1) and hence in liver fat content. Starvation increased and feeding decreased liver GPT compared with cod at the start of the experiment. Fish in the mixed treatments had low GPT activities, close to values measured in fed cod. To remove the effect of lipid storage on hepatic enzyme activities, GPT was expressed relative to DNA content (Table 2). Expressed in this fashion, mean cellular GPT

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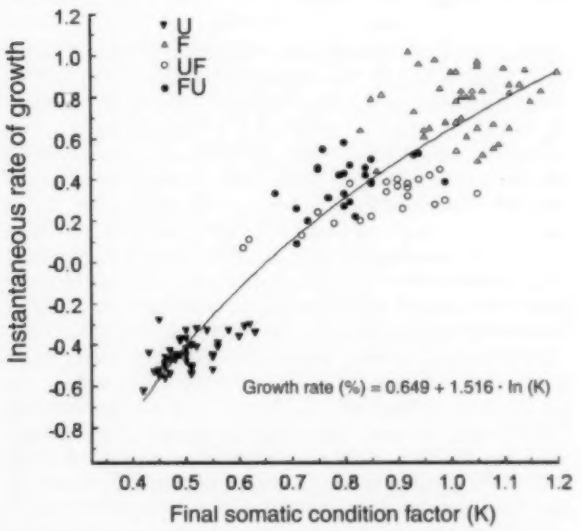
Table 1. Condition factor, liver somatic index, and growth rate of Atlantic cod exposed to one of four experimental treatments over a period of 16 weeks at 10°C.

	Fish size (cm)	Feeding regime				
		I	U	F	UF	FU
Initial condition factor (based on total mass)		0.71±0.11*a* (20)	0.75±0.06*a* (45)	0.74±0.09*a* (47)	0.74±0.07*a* (23)	0.72±0.08*a* (23)
Initial liver somatic index	<40	2.53±1.41 (9)				
	>40	3.17±1.29 (11)				
Final condition factor (based on somatic mass)	<40		0.49±0.04*a* (21)	1.02±0.09*b* (24)		
	>40		0.51±0.06*b* (24)	1.04±0.09*b* (23)	0.87±0.11*c* (23)	0.81±0.08*c* (23)
Final liver somatic index	<40		0.54±0.08*a* (21)	6.58±1.23*b* (24)		
	>40		0.61±0.08*a* (24)	7.60±1.31*b* (23)	6.18±1.57*c* (23)	3.84±1.72*d* (23)
Growth rate over 16 weeks (%)	<40		–0.50±0.06*a* (21)	0.81±0.14*b* (24)		
	>40		–0.40±0.07*a* (24)	0.71±0.13*b* (23)	0.30±0.10*c* (23)	0.38±0.12*c* (23)
Growth rate final 8 weeks	<40		Negative	Positive		
	>40		Negative	Positive	Positive	Negative

Note: I, values at the start of the experiment; U, not fed; F, fed to satiation three times weekly; UF, not fed during 8 weeks and then fed to satiation during 8 weeks; FU, fed to satiation during 8 weeks and then not fed during 8 weeks. Values are means ± SD (*n* in parentheses). Means followed by the same letter within rows are not significantly different ($P > 0.01$). Growth rates over the final 8 weeks were not measured; fish were categorized as having had a positive or a negative growth rate during that period.

Fig. 1. Relationship between overall rate of growth during the experiment and final somatic condition factor in Atlantic cod exposed to one of four experimental treatments over a period of 16 weeks at 10°C. U, not fed; F, fed to satiation three times weekly; UF, not fed during 8 weeks and then fed to satiation during 8 weeks; FU, fed to satiation during 8 weeks and then not fed during 8 weeks.



activity in liver followed a similar pattern as for mean cellular LDH activity in muscle, i.e., low activities in U cod and high activities in F cod. However, expressed in this form, differences in mean cellular LDH or GPT activities of FU and UF cod disappeared. This suggests that the differences in mass-specific activities between FU and UF were accompanied by differences in cell size. Fish size had no significant effect on the activity of liver GPT expressed on a wet mass or DNA content basis ($P > 0.01$).

White muscle protein contents and buffer capacities were influenced by feeding regime (Table 3). F cod had significantly higher muscle protein contents and buffer capacities than U cod. No significant difference was found, however, between mixed treatments. Cod in the mixed treatments had similar myofibrillar protein contents, slightly lower sarcoplasmic protein contents, and slightly higher buffer capacities than F cod. Size did not influence protein contents and buffer capacities. Differences in muscle LDH activities between treatments followed the same pattern when enzyme activity was expressed relative to myofibrillar or sarcoplasmic proteins contents as when expressed on a wet mass basis (Tables 2 and 3), and fish size had no influence ($P > 0.01$).

Feeding during 16 weeks (F) resulted in a low RNA:DNA ratio in white muscle, while refeeding following a period of starvation (UF) resulted in a significantly higher RNA:DNA ratio (Table 3). The RNA:DNA ratio in unfed (U) or recently unfed (FU) cod was not significantly different from that in cod sampled at the start of the experiment. RNA concentration was higher at the start of the experiment and in UF cod than in FU, U, and F cod. The low RNA:DNA ratio in F cod resulted from a significantly lower RNA concentration. The high RNA:DNA ratio in recently fed (UF) cod, on the other hand, resulted from an increased RNA concentration. Fish size had no significant influence on DNA and RNA concentration or on the RNA:DNA ratio ($P > 0.01$; Table 3).

Discussion

The design of the present study provided an opportunity to determine which variables were correlated with energetic condition and which were correlated with recent growth rates in cod. While final condition factor and growth rates were correlated when growth rates were calculated for the whole experimental period (16 weeks), no correlation was expected for cod in the mixed treatments during the second half of the

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Table 2. CCO activity in the intestine, LDH activity in the white muscle, and GPT activity in the liver of Atlantic cod exposed to one of four experimental treatments over a period of 16 weeks at 10°C.

	Feeding regime				
	I	U	F	UF	FU
CCO (units·g wet mass^{-1})	6.80±0.91*a* (20)	4.14±1.03*b* (45)	9.43±1.29*c* (45)	9.53±1.14*c* (23)	6.92±1.10*a* (23)
LDH (units·g wet mass^{-1})	73.6±36.3*a* (20)	16.3±7.9*b* (45)	188.8±43.7*c* (46)	170.2±45.0*c* (23)	120.1±34.7*d* (22)
LDH:DNA (units·μg^{-1})	0.59±0.30*a* (20)	0.14±0.06*b* (31)	2.00±0.61*c* (45)	1.57±0.38*d* (23)	1.41±0.51*d* (22)
GPT (units·g wet mass^{-1})	17.74±6.51*a* (20)	24.50±8.16*b* (27)	8.10±1.93*c* (47)	11.48±3.19*d* (23)	9.50±6.08*c* (23)
GPT:DNA (units·μg^{-1})	0.031±0.024*a* (20)	0.023±0.016*a* (26)	0.111±0.033*b* (47)	0.075±0.036*c* (23)	0.071±0.029*c* (23)

Note: I, values at the start of the experiment; U, not fed; F, fed to satiation three times weekly; UF, not fed during 8 weeks and then fed to satiation during 8 weeks; FU, fed to satiation during 8 weeks and then not fed during 8 weeks. Values are means ± SD (*n* in parentheses) and are expressed as micromoles of substrate converted to product per minute. Means followed by the same letter within rows are not significantly different ($P > 0.01$). The values for I, U, and F are the pooled results for the two size-classes, as size-classes did not differ significantly ($P > 0.01$).

Table 3. White muscle protein contents, nucleic acid contents, and buffer capacity of Atlantic cod exposed to one of four experimental treatments over a period of 16 weeks at 10°C.

	Feeding regime				
	I	U	F	UF	FU
Myofibrillar proteins (mg·g wet mass^{-1})		57.6±13.0*a* (18)	89.1±13.3*b* (26)	84.7±15.3*b* (15)	91.4±11.5*b* (14)
Sarcoplasmic proteins (mg·g wet mass^{-1})		16.3±8.6*a* (18)	37.2±5.9*b* (26)	32.9±9.9*bc* (15)	29.7±4.6*c* (14)
Buffer capacity (β·g wet mass^{-1})[a]		36.6±6.3*a* (17)	61.6±9.4*b* (28)	66.1±8.4*c* (15)	67.4±6.1*c* (15)
DNA (μg·g wet mass^{-1})	127.4±25.3*a* (20)	116.2±34.5*a* (31)	97.2±16.4*b* (45)	109.2±16.7*ab* (23)	88.1±14.5*b* (23)
RNA (μg·g wet mass^{-1})	1138±259*a* (20)	827±303*b* (31)	572±164*c* (45)	1332±343*a* (23)	700±201*bc* (23)
RNA:DNA	9.02±1.50*a* (20)	7.59±3.06*a* (31)	5.91±1.47*b* (45)	12.23±2.68*c* (23)	8.21±2.76*a* (23)

Note: I, values at the start of the experiment; U, not fed; F, fed to satiation three times weekly; UF, not fed during 8 weeks and then fed to satiation during 8 weeks; FU, fed to satiation during 8 weeks and then not fed during 8 weeks. Values are means ± SD (*n* in parentheses). Means followed by the same letter within rows are not significantly different ($P > 0.01$). The values for I, U, and F are the pooled results for the two size-classes, as size-classes did not differ significantly ($P > 0.01$).

[a]β, slykes (micromoles of NaOH that must be added to increase the pH of 1 g of muscle by 1 pH unit).

experiment; cod in the mixed treatments ended with similar condition factor values while having opposite growth rate trajectories (positive in UF cod and negative in FU cod). Simple condition indices such as the condition factor and the liver somatic index are inversely correlated with tissue water contents and positively correlated with both specific and total energy contents in cod (Lambert and Dutil 1997*a*). The discrimination between energetic condition and growth rates demonstrates that LDH activity in white muscle and CCO activity in the intestine are the only variates that reflected short-term changes in growth rates. Other variates more adequately reflected energetic condition.

Only CCO activity in the intestine consistently matched growth rates during the second half of the experiment. Not only were significant differences found between mixed treatments, but CCO levels returned to initial levels in fish that were fed 8 weeks and then starved for 8 weeks. Responses to feeding regime took place within a period of 8 weeks. This confirms the observation made by Goolish and Adelman (1987) and Pelletier et al. (1994) who found that CCO activity of the gastrointestinal tract increased two- to four-fold in faster growing fish. Goolish and Adelman (1987) hypothesized that this increase was due to increased protein synthesis associated with the increase in the relative size of the gut in growing largemouth bass (*Micropterus salmoides*). This cannot explain the increased CCO activity observed in the present study, as the relative mass of the intestine was not influenced by growth rate in cod (Pelletier et al. 1994), unless these proteins would be produced to allow an increased turnover of proteins in the intestine of rapidly growing fish. Conversion efficiency, on the other hand, increased with increased intestinal CCO activity in the latter study, suggesting that CCO response might mediate changes in digestive and absorptive capacity which in turn result in increased growth rates. Goolish and Adelman (1987) also observed that the processing ability of the gastrointestinal tract was maximal in the intermediate-ration group, i.e., in fish that devoted a larger proportion of their whole-body CCO activity to the gastrointestinal tract. These results suggest that CCO activities in the intestine of wild fish should not be interpreted in terms of instantaneous growth rates but rather in terms of the capacity of fish to take advantage of ingested food.

LDH activity in white muscle of cod differed among treatments (Table 2), but did not consistently match changes in growth rates or energetic condition. LDH activity matched growth rates of cod with positive growth rates (within 8 weeks). However, LDH activity was higher in FU cod than in C cod, suggesting that reductions of LDH activity in muscle take place slowly compared with reductions of CCO activity in the intestine and do not reflect declining growth rates (within 8 weeks) adequately. Sullivan and Somero (1983) demonstrated that ration size can influence the activity of a glycolytic enzyme (LDH) and first proposed that enzyme activity might be used to estimate the nutritional state of wild fish. LDH activity was correlated significantly with growth rates in pollock after 2 weeks of exposure to different feeding regimes (Mathers et al. 1992) and in cod after 6 weeks (Pelletier et al. 1994), 12 weeks (Pelletier et al. 1993*b*), and 16 weeks (this study). The lack of a relationship between LDH

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activity and RNA levels, while mitochondrial enzymes were significantly correlated with RNA levels, led Mathers et al. (1992) to conclude that LDH was not directly related to feeding metabolism. Increased LDH activity in fish with faster growth rates may rather reflect the improved energetic status. Further, larger fish tend to defend higher muscle levels of LDH due to the higher power requirements of burst swimming (Somero and Childress 1980).

Because fish in the mixed treatments reached similar values of the GPT:DNA ratio in the liver at the end of the experiment, we conclude that GPT activity does not reflect growth rates, but rather reflects changes in feeding regime and resulting changes in energetic condition. Liver GPT activity was considerably influenced by liver fat content, as shown by differences in the liver somatic index, as the liver is a major site for the storage of lipids in cod (Dutil et al. 1995; Lambert and Dutil 1997*a*). Foster and Moon (1991) recommended expressing hepatic enzyme activity relative to tissue DNA content. Cod showing positive and negative growth rates during the final 8 weeks did not differ in their GPT:DNA ratio (Tables 1 and 2). However, the GPT:DNA ratio was very low in cod starved during 16 weeks and was highest in cod fed to satiation during the same period. Thus the GPT:DNA ratio is not adjusted to growth rate, but may rather respond to more extreme situations, i.e., low activities in livers with minimal specific lipid reserves and high activities in livers with maximal specific lipid reserves. Pelletier et al. (1994) found a positive relationship between the GPT:DNA ratio and growth rates in both cod with negative growth rates and cod with positive growth rates. They suggested that such increases in GPT:DNA ratios in faster growing fish reflected enhanced oxidation of amino acids not required for protein synthesis. GPT:DNA ratios could also increase in fish that continue building up energy reserves once liver size has increased to a maximum, given requirements for transaminations to form the necessary amino acids. The liver responds to changes in growth rates by an adjustment of its total mass (Black and Love 1986). The GPT:DNA ratio could be envisaged as an indicator of the duration of starvation (low values) or prolonged maintenance of large lipid reserves (high values).

Many recent studies have shown that enzymatic activities in various tissues of fish are correlated with growth rates. Goolish and Adelman (1987) suggested a positive correlation between growth rates and CCO activity in the musculature of largemouth bass, but further studies have shown that mitochondrial enzyme activities in muscle are in general poorly or not significantly correlated with growth rates (Goolish and Adelman 1987; Cai and Adelman 1990; Kiessling et al. 1991; Mathers et al. 1992; Pelletier et al. 1993*a*, 1994). Enzyme activities in these studies were hypothesized to be adjusted to meet growth requirements, namely producing enough energy in the form of ATP to fuel the increased rate of protein synthesis of rapidly growing fish, particularly in white muscle, a major site of protein synthesis (Houlihan et al. 1988). Blier et al. (1997), however, determined that cod do not specifically increase the aerobic capacity of white muscle to match growth rates. They estimated that potential daily rates of ATP synthesis in white muscle were considerably higher than required by protein synthesis in spite of the fact that mitochondrial density is low in white muscle (Johnston and Moon 1981), the site of ~30% of whole-body synthesis (Houlihan et al. 1988). In the intestine, on the other hand, mitochondrial enzymes would be indirectly related to growth rates. CCO activity in the intestine reflects conversion ratio, possibly through production of the ATP required for the synthesis of digestive enzymes or active transport of nutrients across the intestinal membrane (Pelletier et al. 1994). Stronger and more consistent correlations between glycolytic enzymes and growth rates (Pelletier et al. 1993*b*, 1994) are unlikely to reflect a limiting role of glycolysis for protein synthesis, but rather reflect the increased deposition of the metabolic machinery required for the activity of white muscle. Sullivan and Somero (1983), Pelletier et al. (1993*b*), and Guderley et al. (1996) suggested that faster growing fish would have a higher capacity for glycolytically powered bursts of locomotion. The present study supports the conclusions reached by Pelletier et al. (1994) and shows that CCO activities in the intestine of cod match growth trajectories and do not reflect energetic condition. LDH activity of white muscle also responded to changes in growth rates, but LDH activity in muscle did not decline as rapidly as CCO activity in the intestine when growth rates declined, again indicating that LDH levels are the result of growth and not a requirement for growth.

Protein concentrations and buffer capacities did not show any marked relationship to growth rates. They were influenced by prolonged starvation, but not by inversion of the feeding regime. Sarcoplasmic proteins varied between feeding regimes but did not reflect growth rates or energetic condition consistently. The low concentrations of sarcoplasmic proteins observed in starved fish in our study and in Guderley et al. (1996) are consistent with Houlihan et al.'s (1988) conclusion that axial muscle, besides its role in swimming, may serve as a source of energy and amino acids for various tissues. Myofibrillar proteins, on the other hand, do not respond rapidly to changes in food availability. Myofibrillar proteins are more closely related to swimming requirements and thus would not appear to be used as an energy store unless other energy stores are depleted. Red muscle fibres retain their integrity longer than white muscle fibres during starvation in cod (Black and Love 1986); nevertheless, prolonged starvation resulted in substantial atrophy of both slow and fast muscle fibres in pollock (Beardall and Johnston 1985). Myofibrillar proteins and buffer capacities were only affected by prolonged food deprivation in our study. This lack of response of structural proteins and buffer capacities after 8 weeks of food deprivation may allow cod to maintain their locomotory abilities in periods of low food availability and thus allow them to benefit more from short periods of greater food availability. The coordinated changes in the glycolytic apparatus with changes in growth rates observed in a comparison of starved and fed cod (Guderley et al. 1996) were not fully apparent in our study. We found that LDH activity and sarcoplasmic proteins followed similar patterns in the different treatments whereas buffer capacities did not. Buffer capacities were lower in continuously starved than fed cod but did not respond in cod exposed to shorter periods of food deprivation. Buffer capacities in cod of the mixed treatments were higher, in fact, than in cod fed to satiation during 16 weeks in our study, but levels reached were considerably lower (65 $\beta \cdot g^{-1}$) in our study than in fed or wild cod (96 $\beta \cdot g^{-1}$) in Guderley et al. (1996).

The response of nucleic acids did not consistently reflect growth trajectories or energetic condition in our study. There

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is a positive correlation between growth rates and protein synthesis in white muscle, and the RNA:protein ratio is correlated with rates of protein synthesis (Loughna and Goldspink 1984; Houlihan et al. 1993). Thus, higher growth rates are expected to increase RNA concentrations and RNA:DNA ratios. Mathers et al. (1992) and Foster et al. (1993) found significant correlations between RNA concentrations and both positive and negative growth rates. RNA concentrations responded within 2 weeks (Mathers et al. 1992). In cod, RNA concentrations and RNA:DNA ratios in muscle were significantly reduced after 6 weeks of starvation, but did not correlate significantly with positive growth rates (Pelletier et al. 1994, 1995). Pelletier et al. (1994, 1995) suggested that cod may increase their growth rate by increasing RNA activities and not RNA concentrations. Different results may occur over longer periods. Prolonged starvation followed by refeeding resulted in a much higher RNA:DNA ratio than in continuously fed cod (Black and Love 1986), with significantly higher ratios being reached after 60 days and maintained to the end of the experiment (200 days). Fish fed following 8 weeks of starvation (UF) in our study reached condition factors below 0.70 by the time they were refed. They were probably growing more rapidly during the second half of the experiment than cod fed to satiation during 16 weeks (compensatory growth), which could explain an increased RNA concentration in the mixed treatment. Both DNA and RNA concentrations were lower in unfed cod (U and FU) than in recently fed cod (UF), which could have resulted from a depressed rate of protein synthesis, as food was not available. Lower RNA concentrations and RNA:DNA ratios in continuously fed cod than in unfed (U) and recently unfed (FU) cod, however, are contradictory. Continuously fed cod in our study never experienced poor condition factors. Higher RNA:DNA ratios in U and UF cod may possibly have been brought about by a requirement to produce more proteins as condition deteriorated below a threshold level. While our results suggest that feeding history may be a major factor determining the response of nucleic acids, they are not readily explained by the experimental protocol and did not reflect overall growth rates and final energetic condition.

Size did not have a significant effect on enzyme activities and nucleic acid concentrations in our study. Enzyme activities (Somero and Childress 1980; Goolish 1991; Pelletier et al. 1993*a*, 1994) and nucleic acid concentrations (Buckley and Lough 1987; Mathers et al. 1992) were correlated with size in several fish species. Two size-classes were examined in the present study. Differences in size were not great, however, considering the range in size for the species, and variability within each size-class was high. This could have masked potential differences in enzyme activities and nucleic acid concentrations. Size-class effects were nearly significant for muscle DNA, muscle LDH, and muscle LDH:DNA ratio. Not detecting any size effect in our study does not indicate that size has no effect on enzyme activities and nucleic acid concentrations. It likely reflects the fact that allometric effects are not strong within the narrow range of sizes examined (Mathers et al. 1993).

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Influence of fish density on the energy allocation pattern of juvenile brook trout (*Salvelinus fontinalis*)

François Marchand and Daniel Boisclair

Abstract: Our objectives were (*i*) to assess the influence of fish density on the energy allocation pattern of juvenile brook trout (*Salvelinus fontinalis*) and (*ii*) to define the mechanism by which this influence occurs. We estimated growth, consumption, and activity rates of brook trout held in 8-m^3 enclosures characterized by two different densities (four or eight fish per enclosure; D4 and D8 enclosures, respectively). Eight experiments designed to estimate these variables were performed during a 27-day period. Fish from D4 enclosures ($\Delta\overline{W} = 0.46$ g) grew twice as much as those from D8 enclosures ($\Delta\overline{W} = 0.24$ g). For any given experiment, consumption rates were not significantly different between the two fish densities (0.434–1.09 g dry·100 g wet^{-1}·day^{-1}). However, fish stocked in D8 enclosures displayed more aggressive behavior, executed 5.5 times more movements, and swam at speeds 13% faster than fish in D4 enclosures. These differences resulted in activity rates fourfold higher in D8 enclosures than in D4 enclosures. Empirical analyses combining our results with published values of growth, consumption, and activity rates supported the hypothesis that competition can have a negative effect on growth through a decrease in consumption rates and an increase in activity costs.

Résumé : Les objectifs de notre étude étaient de (*i*) déterminer l'influence de la densité de poissons sur le patron d'allocation énergétique chez des juvéniles d'omble de fontaine (*Salvelinus fontinalis*) et (*ii*) de déterminer le ou les mécanismes spécifiques à cette influence. Nous avons estimé la croissance, la consommation et le taux d'activité d'ombles de fontaine gardés dans des enclos de 8 m^3, caractérisés par deux densités de poissons différentes (quatre ou huit poissons par enclos; enclos D4 et D8, respectivement). Sur une période de 27 jours, huit expériences ont été faites pour évaluer ces différentes variables pour les deux densités de poissons. Les ombles de fontaine de l'enclos D4 ($\Delta\overline{W} = 0{,}46$ g) ont eu une croissance deux fois plus importante que les ombles de l'enclos D8 ($\Delta\overline{W} = 0.24$ g). Pour chacune des expériences, les taux de consommation journalière n'ont pas varié de façon significative entre les deux densités (de 0,434 à 1,09 g sec·100 g $humide^{-1}$·$jour^{-1}$). Cependant, les ombles introduits dans les enclos D8 ont exhibé un comportement plus agressif, exécutant 5,5 fois plus de mouvement d'agression et nageant à des vitesses 13% plus rapides que les ombles dans les enclos D4. Ces différences ont résulté en un taux d'activité quatre fois plus grand dans les enclos D8. Une analyse empirique combinant nos résultats à des valeurs de croissance, de consommation et d'activité déjà publiés supporte l'hypothèse que la compétition peut avoir une influence négative sur la croissance des poissons par la diminution des taux de consommation et par l'augmentation des coûts énergétiques associés à l'activité.

Introduction

The growth rate by weight of individuals from a given fish species can vary up to 10-fold among populations (Boisclair and Leggett 1989*a*; Rubin 1993). Many abiotic and biotic factors, such as water temperature (Jobling 1983) and the quantity or quality of prey (Brooks and Dodson 1965; Hart and Connellan 1984), have been used to explain that variability. One of the most studied factors is fish density (number of fish per cubic metre; Magnuson 1962; Jobling and Reinsnes 1986) because it is commonly estimated in the field (directly or using catch per unit of effort data) and easy to manipulate under laboratory or experimental conditions. Furthermore, fish density is expected to have a direct influence on prey availability (Hanson and Leggett 1985; Mittelbach 1988).

Many laboratory and field studies have shown that an increase in fish density generally results in a decrease in individual growth rate (Jørgensen et al. 1993; Mazur et al. 1993). However, there appears to be controversy regarding the mechanisms by which this effect occurs. For instance, Hanson and Leggett (1985) manipulated fish density in enclosures and assessed its influence on the growth rates of pumpkinseed (*Lepomis gibbosus*) and yellow perch (*Perca flavescens*). They found a negative relationship between fish density and growth rates of yellow perch. They did not directly estimate food consumption or activity rates but interpreted that fish density affected fish growth via its influence on consumption rates (i.e., exploitative competition). Boisclair and Leggett (1989*b*) assessed the influence of a series of environmental conditions on the growth rates of three year-classes of yellow perch from 12 populations. The most consistent relationship revealed by their work was a negative relationship between fish growth and fish density. However, they did not find a relationship between

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food consumption and fish density. Combination of their data on fish growth and consumption rates in a bioenergetic model allowed them to estimate activity rates by difference. Activity costs ranged from 0 to 40% of yellow perch energy budget (Boisclair and Leggett 1989*c*). Taken together, their work suggests that fish density influences fish growth, not necessarily by decreasing food consumption but also by increasing metabolic expenditures through, for instance, higher activity rates. This interpretation, which has been the subject of debate (Hayward 1990; Hewett et al. 1991), supports the suggestion that social interactions, which may increase in number and in intensity with fish density, may play a major role in explaining variations in growth rates (Jobling 1985; Jobling and Reinsnes 1986).

Our objectives were (*i*) to assess the influence of fish density on fish growth rates and (*ii*) to determine the mechanisms by which this influence occurs. These objectives were achieved by estimating energy acquisition (consumption rates) and allocation patterns (growth versus activity rates) in juvenile brook trout (*Salvelinus fontinalis*) held at different densities.

Methodology

Experimental design

We assessed the influence of fish density on their growth, consumption, and activity rates by performing a series of experiments on brook trout kept in in situ enclosures. The study was conducted in Lake Long, a mesotrophic lake at the Station de Biologie des Laurentides de l'Université de Montréal (SBLUM) in the lower Laurentian region of Québec. We moored twelve 8-m^3 enclosures ($2 \times 2 \times 2$ m, 6-mm mesh size) on the 4-m isobath of the lake. The mesh size allowed zooplankton (the only prey available to fish inside our enclosures) to move freely in and out of the enclosures. The bottom of each enclosure was 1 m from the bottom of the lake and the top of the enclosure was 1 m below the surface. Each enclosure was equipped with a bidirectional pump system. This system allowed the estimation of prey availability inside the enclosures at regular intervals during our study.

A total of 1000 brook trout belonging to a homogeneous genetic stock were purchased at a local hatchery in mid-May 1993. These fish (further referred to as the original stock) were kept in aquaria at the SBLUM. On June 1, 1993, we introduced four 0+ brook trout (average mass = 0.64 g wet) into six enclosures (enclosures 4-1 to 4-6, further referred to as D4 enclosures) and eight 0+ brook trout (average mass = 0.68 g wet) into the other enclosures (enclosures 8-1 to 8-6, further referred to as D8 enclosures). These experimental densities were selected because they represented fish densities that may be found under natural conditions (see Discussion) and because they allowed the estimation of confidence intervals around the variables that represented the focus of our study. Enclosures 4-1 and 8-1 were equipped with a pair of underwater videocameras (Boisclair 1992). The videocameras were oriented to observe fish moving in different parts of our enclosures such as the center and the edges. When the visibility was sufficient, acitivity in the corners could also be estimated. The two enclosures containing the videocamera systems were not manipulated or moved during the complete duration of our study (27 days). A total of eight experiments were performed at 3-day intervals during that period (experiments 1–8). Each experiment consisted of the estimation of the consumption and activity rates of the fish held at two densities.

Experimental procedure

Growth rate

Growth rates were estimated by weight using the brook trout inside enclosures 4-1 and 8-1. Each brook trout stocked inside these enclosures was weighed with an electronic balance (±0.01 g) and marked by fin clipping at the beginning of the study. These fish were never manipulated during the study. Initial caloric density of the fish was determined using 10 brook trout sampled at random from the original stock at the beginning of the study. These fish were dried at 60°C for 48 h and their caloric densities (1 cal = 4.185 J) were estimated individually using a Phillipson microbomb calorimeter. Brook trout from enclosures 4-1 and 8-1 were collected at the end of the study and their weight and caloric density were determined using the same procedure.

Consumption rate

Consumption rates of fish stocked inside D4 and D8 enclosures were estimated using enclosures 4-2 to 4-6 and 8-2 to 8-6. Two enclosures (D4 and D8) were sampled at 4- to 5-h intervals (06:00, 11:00, 16:00, 20:00, and 24:00). All fish from these enclosures were measured, weighed, and dissected within 1 h of sampling. The complete digestive tract content was dried (60°C for 24 h) and weighed (±0.00005 g) individually. Fish collected from each enclosure were replaced immediately following sampling by the same number of fish selected from the original stock. These fish were used to estimate consumption rate during the next experiment.

Activity rate

Activity rates of brook trout were estimated by filming the movements of the fish inside enclosures 4-1 and 8-1 for 1 h at 3- to 4-h intervals during daylight hours only (07:00, 10:00, 12:00, 15:00, and 19:00). We used the stereocinematographic method to estimate the *X*, *Y*, *Z* Cartesian coordinates (Boisclair 1992). Fish position was estimated at 1-s intervals using an image analysis system (Jandel Video Analysis Software). The coordinates were further transformed in terms of fish speed, acceleration, and turning rates following the procedure described by Tang and Boisclair (1993).

Environmental conditions

Environmental conditions inside our enclosures were defined as water temperature and zooplankton biomass, taxonomic composition, and size structure. Water temperature and zooplankton biomass were monitored during each experiment. Water temperature was taken in the center of the area occupied by our enclosures. Water temperature was determined at 0.5-m intervals from 1 m below the surface to 1 m above the bottom of the lake with an electronic thermometer (YSI, model 57, ±0.1°C). Zooplankton biomass (±0.00005 g dry) was estimated by pumping ten 10-L samples of water from each enclosure. Zooplankton were killed by immersion in carbonated water following sampling. Water samples were filtered on preweighed (± 0.000 05 g·dry) 1-µm pore size fiberglass filters and dried at 60°C for 3 h. Following the sampling of zooplankton, a similar volume of unfiltered lake water was pumped into each enclosure. Zooplankton size and taxonomic composition were estimated on three occasions (experiments 2, 5, and 8) by pumping an additional 10 L of water from each enclosure. Zooplankton in the eleventh samples (one for D4 and one for D8 enclosures) were killed in a 4% sucrose–formalin solution. Zooplankton were analysed in the laboratory using a microscope (Wild Heerbrugg M4 at 40× magnification) equipped with a graduated ocular. One hundred individuals from each sample were measured (±0.005 mm) and identified.

Computations

Growth rate

Individual growth of the fish stocked inside enclosure 4-1 or 8-1 was estimated independently as the difference between the mass of each fish at the beginning (June 1; W_i, grams wet) and the end (June 27; W_e, grams wet) of our study. Caloric growth was estimated on an

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individual basis as the difference between the product of fish mass and its caloric density at the beginning and the end of the study.

Consumption rate

The quantity of food consumed by fish during 1 day (Q, grams dry per 100 g wet per day) was estimated using the Eggers (1977) model:

$$(1) \quad Q = \overline{F} \cdot R \cdot 24$$

where $\overline{F}$ is the mean gut fullness of the fish sampled during 1 day (geometric mean, grams dry of complete digestive tract per 100 g wet of body), R is evacuation rate (per hour), and 24 is the number of hours in 1 day.

Evacuation rates were taken from values estimated for 0+ brook trout held under laboratory conditions at temperatures ranging from 18 to 21°C (Boisclair and Sirois 1993; Sirois and Boisclair 1995). Evacuation rates were defined as the slope of the relationship between the natural logarithm of gut fullness and the time since feeding.

Average daily energy intake by individual fish (C, calories per day) was estimated as

$$(2) \quad C = \frac{Q \cdot \overline{W} \cdot \mathrm{CDP}}{100}$$

where $\overline{W}$ (grams wet) is the expected mean weight of the fish inside enclosure 4-1 or 8-1 and CDP is the caloric density of zooplankton. Prey caloric density was estimated using data taken from Cummins and Wuycheck (1971). Expected mean weight of the fish inside an enclosure (4-1 or 8-1) was estimated with a linear interpolation between initial and final fish weight inside the enclosure. Total energy intake for the complete duration of our study was calculated as the area under the curve between our eight estimates of average energy intake.

Activity rate

The videocameras we used to observe fish do not allow identification of individual fish and sampled only a maximum of 3.6 m^3 of our 8-m^3 enclosures. The procedure we adopted to estimate fish activity costs is therefore not adequate to determine the activity rate of a specific fish. We circumvented this difficulty by estimating the quantity of energy spent by an average individual using total activity rates of all fish inside an enclosure during a given period. This calculation constituted three steps. First, we estimated the energy spent swimming by all fish observed within the volume sampled by our videocameras during each hour of filming by transforming speed to energy expenditures using the model of Boisclair and Tang (1993). Second, we extrapolated that value to our complete enclosure. These two first steps resulted in the estimation of total hourly activity rates. Finally, we estimated individual activity rates per hour by dividing total hourly activity rates by the number of fish in an enclosure. A complete description of the computations required to estimate individual activity rates was presented by Boisclair (1992), Boisclair and Sirois (1993), and Sirois and Boisclair (1995).

The only modification applied to estimate activity rates concerned the model used to transform swimming speed to energy expenditures. During our study, we noted that fish inside our enclosure with the highest fish density exhibited aggressive behaviors. The model we used to transform fish swimming speed into energy costs was developed using literature data systematically excluding experiments performed while fish were aggressive (see Boisclair and Tang 1993). Hence, the model we used may underestimate the costs of movements during aggressive behavior. Some studies suggest that aggressive movements require 1.5–4 times more energy than nonaggressive movements at the same speed (Brett 1964; Brett and Sutherland 1965; M. Krohn and D. Boisclair, unpublished data). We therefore corrected our estimates of the costs of swimming at a given speed during aggressive movements by multiplying the values obtained using the model of Boisclair and Tang (1993) by a conservative factor set at a value of 2 (closest value to the mean of costs of aggressive versus nonaggressive behavior). Swimming speed values were estimated using geometrical formulas based on successive fish positions taken at 1-s intervals in a X, Y, Z Cartesian coordinate system (Tang and Boisclair 1993).

Testing of assumptions

The experimental design we adopted to estimate fish growth, activity, and feeding rates assumes that (*i*) consumption and activity rates were similar among all enclosures characterized by the same fish density, (*ii*) activity rates of fish that were inside our enclosures for long periods (as in enclosures 4-1 and 8-1) were comparable with those of fish inside enclosures for only 3 days (as in enclosures 4-2 to 4-6 and 8-2 to 8-6), (*iii*) activity rates during the night (when fish could not be filmed) were negligible, and (*iv*) the visibility, which affected the fraction of the enclosure under observation, did not affect our estimates of activity rates.

The similarity of consumption rates among enclosures was verified by Sirois and Boisclair (1995). They evaluated the variability of the feeding cycle of 0+ brook trout kept in separate enclosures by sampling all the fish in different enclosures at the same time. They found that the feeding behavior of fish stocked inside a group of five enclosures can be adequately represented by that of fish inside one enclosure. We assessed among-enclosure variations in activity rates by simultaneously filming fish kept inside two enclosures under the same environmental conditions and comparing activity estimates. Furthermore, we also compared hourly activity rates of fish that had been inside an enclosure for 3 and 27 days. Finally, we assessed the influence of visibility on activity rates by testing the existence of a relationship between activity rates and the proportion of the enclosure that was under observation. Our assumption that fish activity rates were negligible at night was based on our observation that 0+ brook trout do not feed inside our enclosures at night and on the hypothesis of the existence of a positive relationship between activity and consumption rates (Kerr 1982). This hypothesis was supported by Boisclair (1992) who showed that there is a positive correlation between activity costs and consumption rates for brook trout kept in enclosures. Furthermore, the validity of this hypothesis was supported by Boisclair and Sirois (1993) and Sirois and Boisclair (1995) who successfully modeled the growth of brook trout inside enclosures using this assumption.

Statistical analyses

We used paired t-tests to analyse the significance of the variation of fish weight. One-way analyses of variance were used to test the significance of the variation of fish weight and caloric content between the two densities. The significance of the variations in consumption rates, zooplankton biomass, and hourly and daily activity rates was evaluated with two-way analysis of variance. As required by the assumptions of parametric tests, fish digestive tract content and zooplankton biomass were normalized by natural logarithmic transformation. Because of the large number of data and unequal sample size, variations in swimming characteristics were analysed using permutations and two-way analysis of variance (Manly 1991). Swimming characteristics were also presented and compared as percentages. Significance of variations in zooplankton size structure was assessed using a Mann–Whitney nonparametric test.

Results

Growth rate

There was no significant difference ($t_{10} = -0.23$, $p > 0.80$) between the initial mean mass of the fish stocked inside enclosures 4-1 and 8-1 (Table 1). Mean caloric density of the fish at the beginning of our study was 4450 cal·g dry^{-1} (SD = 707). The weight of the fish held at the two experimental densities increased during the 27-day period (enclosure 4-1: $t_3 = -7.95$,

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Table 1. Individual fish weight at the beginning (June 1, 1993) and the end (June 27, 1993) of the study.

Fish No.	Total weight (g wet) Initial	Final	ΔW	Final caloric content (cal·g dry^{-1})
Density 4				
1	0.39	0.69	0.30	4591
2	0.48	0.93	0.45	5170
3	0.63	1.13	0.50	4261
4	0.80	1.37	0.57	4693
Average	0.58	1.03	0.46	4679
SD	0.18	0.29	0.11	376
Density 8				
1	0.38	0.39	0.01	4758
2	0.43	0.65	0.22	3837
3	0.45	0.61	0.16	3392
4	0.54	0.73	0.19	4240
5	0.58	0.89	0.31	4571
6	0.62	0.98	0.36	5128
7	0.91	1.10	0.19	4590
8	0.91	1.38	0.47	—
Average	0.60	0.84	0.24	4359
SD	0.21	0.31	0.14	587

Note: ΔW represents individual fish growth. Caloric density at the end of the study was estimated for each fish.

$p < 0.01$; enclosure 8-1: $t_7 = -4.83$, $p < 0.01$). However, the mean individual growth of the fish in enclosure 4-1 (0.46 g wet) was twice that of fish in enclosure 8-1 (0.24 g wet), a significant difference ($t_{10} = 2.66$, $p < 0.025$). Mean caloric density of the fish at the end of our study (Table 1) was not different from initial values (enclosure 4-1: $t_{12} = 0.60$, $p > 0.55$; enclosure 8-1: $t_{15} = -0.28$, $p > 0.75$). Average growth of individual fish between the beginning and the end of our study was 452 cal in enclosure 4-1 and 198 cal in enclosure 8-1 (Table 2).

Consumption rate

Consumption rates of the fish ranged from 0.47 to 1.16 g dry·100 g wet^{-1}·day^{-1} in the D4 enclosures and from 0.45 to 1.1 g dry·100 g wet^{-1}·day^{-1} in the D8 enclosures. Consumption rates estimated during the complete duration of our study (Fig. 1) did not vary among densities ($F_{1,455} = 0.214$, $p > 0.60$), but they did vary among experiments ($F_{7,455} = 11.08$, $p < 0.001$). The interaction term was not significant ($F_{7,455} = 0.909$, $p > 0.45$). These analyses suggest that during any given experiment, individual fish consumed the same quantity of food (within ±14%) regardless of their density. Average energy intake by individual fish ranged from 19.5 to 49.4 cal·day^{-1} inside the D4 enclosures. Corresponding values for the D8 enclosures were 15.3 and 41.6 cal·day^{-1}. Total energy consumption by fish inside the D4 enclosures (800 cal) was 15% higher than that by fish inside the D8 enclosures (698 cal) (Table 2).

Activity rate

The quantity of movements observed during a specific 1-h period ranged from 7 to 328 fish-seconds·m^{-3}·h^{-1} for enclosure 4-1 and from 395 to 767 fish-seconds·m^{-3}·h^{-1} for enclosure 8-1 (Table 3). The number of movements executed by fish inside enclosure 8-1 (mean = 545 fish-seconds·m^{-3}·h^{-1}) was, on average, 5.5 times larger than that for fish at the lower density (mean = 100 fish-seconds·m^{-3}·h^{-1}). Furthermore, aggressive pursuits and interactions were three times more numerous in enclosure 8-1 (mean = 16·m^{-3}·h^{-1}) than in enclosure 4-1 (mean = 5·m^{-3}·h^{-1}).

Mean swimming speed of the fish filmed in enclosure 4-1 ranged from 4.5 to 8.3 cm·s^{-1} (experiments 8 and 5). Fish inside enclosure 8-1 swam at a mean speed ranging from 5.4 to 9.4 cm·s^{-1} (experiments 3 and 8). Permutation tests indicated that mean swimming speeds were different among experiments ($p < 0.001$) and between fish densities ($p < 0.001$). The interaction term was also significant ($p < 0.001$). On average, the mean swimming speed observed at the two experimental densities varied by 13%. For six of our seven experiments, mean swimming speed was higher in enclosure 8-1 than in enclosure 4-1 (Table 3).

Mean acceleration rates were always close to zero and did not vary among experiments ($p > 0.40$) or fish densities ($p > 0.10$). Variance of acceleration was, on average, six times larger in enclosure 8-1 than in enclosure 4-1 (Table 3).

Turning rates ranged from 15.6 to 26.3°·s^{-1} (experiments 8 and 2) for the fish stocked inside enclosure 4-1 and from 28.2 to 42.7°·s^{-1} (experiments 6 and 5) for fish observed in enclosure 8-1. These rates differed between experiments ($p < 0.001$) and fish densities ($p < 0.001$). Mean turning rates of fish inside enclosure 8-1 were always higher than those observed in enclosure 4-1 (average difference = 27%).

For fish inside enclosure 4-1, total activity rates per hour ranged from 0 to 2.4 cal·h^{-1} and individual activity rates from 0 to 0.6 cal·h^{-1} (average = 0.13 cal·h^{-1}). Total activity rates per hour (0–16.80 cal·h^{-1}) and individual activity rates (0–2.1 cal·h^{-1}, average = 0.56 cal·h^{-1}) estimated for fish inside enclosure 8-1 were, on average, 4.3 times higher than corresponding values for the lower fish density. Individual activity rates per hour varied significantly between densities ($F_{1,38} = 8.89$, $p < 0.005$) but not among experiments ($F_{7,38} = 1.03$, $p > 0.40$). The interaction term was not significant ($F_{6,38} = 1.35$, $p > 0.25$). Daily activity rate of individual fish in enclosure 4-1 ranged from 0.07 to 4.24 cal·day^{-1} (mean = 1.96 cal·day^{-1}) (Table 2). Values estimated for the fish inside enclosure 8-1 ranged from 5.96 to 10.12 cal·day^{-1} (mean = 7.94 cal·day^{-1}). Daily activity rate of individuals held at a density of eight fish per enclosure was, on average, four times that of fish held at the lower density.

Environmental conditions

Water temperature at a depth corresponding to the middle of our enclosures ranged from 17.0°C (June 3, 1993) to 19.5°C (June 15, 1993) (Table 4). Water temperature variation within an enclosure (bottom to top) was within ±3° of that found in the center of th enclosure. Zooplankton biomass ranged from 0.11 to 0.18 mg dry·L^{-1} in the D4 enclosures and from 0.12 to 0.18 mg dry·L^{-1} in the D8 enclosures (Table 4). Zooplankton biomass varied among experiments ($F_{7,1139} = 16.5$, $p < 0.01$) and fish densities ($F_{1,1139} = 29.1$, $p < 0.001$). The interaction term was also significant ($F_{7,1139} = 3.0$, $p < 0.01$). A posteriori comparisons indicated that the difference in zooplankton biomass between fish densities was most pronounced during experiments 2, 3, and 7 (Fig. 2). During these experiments, enclosures containing eight fish had between 9.4 and 17.2%

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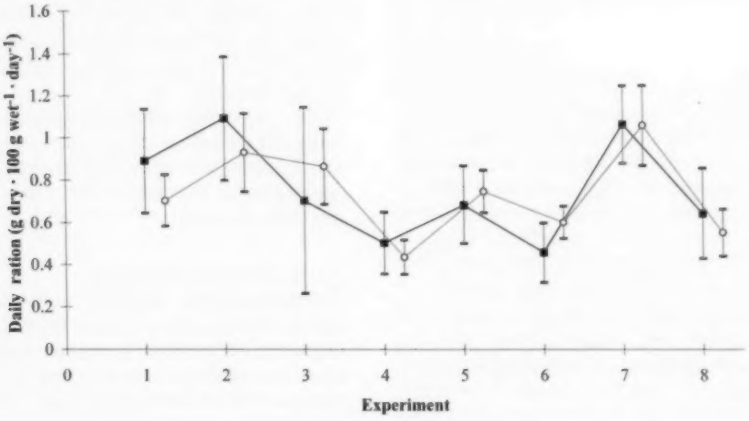
Table 2. Estimated values of fish weight, growth, consumption, and activity rates and other energy expenditures (OEE, estimated by difference) of individual fish held at both experimental densities.

Day	Exp.	Fish weight (g·wet)	Growth (cal·day^{-1})	Consumption (cal·day^{-1})	Activity (cal·day^{-1})	OEE (cal·day^{-1})
Density 4						
0	—	0.58	16.1	25.6	4.2	5.3
3	1	0.63	16.2	27.8	4.2	7.4
6	2	0.68	16.4	36.9	4.2	16.3
9	3	0.73	16.6	25.5	3.4	5.6
12	4	0.78	16.7	19.5	0.2	2.6
15	5	0.83	16.9	28.2	0.1	11.3
18	6	0.88	17.1	20.0	0.4	2.5
21	7	0.93	17.3	49.4	0.4	31.7
24	8	0.98	17.4	33.8	0.5	15.9
27						
Σ(cal·27 days^{-1})			452.1	800.4	52.9	295.4
% of consumption			56		7	37
Density 8						
0	—	0.60	7.5	21.1	7.2	6.5
3	1	0.63	7.5	22.1	7.2	7.4
6	2	0.65	7.4	30.4	7.2	15.8
9	3	0.68	7.4	29.4	7.3	14.7
12	4	0.71	7.3	15.3	7.9	0.1
15	5	0.73	7.3	27.4	9.8	10.2
18	6	0.76	7.3	22.8	7.1	8.5
21	7	0.79	7.2	41.6	10.0	24.3
24	8	0.81	7.2	22.4	6.0	9.3
27		0.84				
Σ(cal·27 days^{-1})			198.3	697.5	209.1	290.1
% of consumption			28		30	42

Fig. 1. Mean daily ration of fish in enclosures containing four (D4 enclosures, solid squares) or eight brook trout (D8 enclosures, open circles). Vertical bars represent 95% confidence intervals.



larger zooplankton biomass than enclosures containing four fish.

The mean size of zooplankton ranged from 0.48 to 0.53 mm for the D4 enclosures and from 0.47 to 0.60 mm for the D8 enclosures (Table 4). Zooplankton size did not vary significantly among experiments or between densities ($-1.88 < Z_2 < -1.13$, $0.06 < p < 0.26$). Copepoda were the principal (68.3%) zooplankton present, followed by Bosminidae (24.9%) and Daphnidae (6.8%). The taxonomic composition of zooplankton did not vary by more than 9% among experiments and fish densities.

Testing of assumptions

Hourly activity rates estimated simultaneously in two

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Table 3. Average number of movements and swimming characteristics of fish held at both fish densities during each experiment.

Exp.	Mean movements (fish-seconds·m^{-3}·h^{-1})	Speed (cm·s^{-1})		Acceleration (cm·s^{-2})		Turning rate (°·s^{-1})	
		Mean	SD	Mean	SD	Mean	SD
Density 4							
1	—	—	—	—	—	—	—
2	328	5.7	2.1	–0.1	2.2	22.2	23.3
3	245	5.6	2.0	–0.2	2.6	26.3	22.7
4	29	4.8	1.7	0.2	1.3	20.9	16.8
5	7	8.3	5.6	0.9	3.2	17.6	10.6
6	21	5.0	1.7	0.1	1.5	18.5	12.6
7	7	4.7	1.6	–0.1	1.7	17.3	10.5
8	59	4.5	2.2	–0.5	2.3	15.6	8.7
Density 8							
1	—	—	—	—	—	—	—
2	767	6.5	5.0	–0.3	5.2	38.0	37.2
3	546	5.4	2.5	–0.4	2.3	30.9	26.9
4	531	6.2	3.7	–0.5	5.0	35.0	32.2
5	516	7.0	5.2	–0.3	5.2	42.7	37.7
6	395	7.4	4.4	–0.4	4.4	28.2	28.5
7	462	7.8	2.8	–0.8	4.3	32.2	26.5
8	599	9.4	14.0	–1.5	4.8	35.2	34.7

Table 4. Zooplankton biomass and size structure inside the enclosures for each experiment and fish densities.

Exp.	Zooplankton						Water temperature (°C)
	Biomass (mg dry·L^{-1})			Size (mm)			
	Mean	SD	*N*	Mean	SD	*N*	
Density 4							
1	—	—	—				17.0
2	0.18	0.12	58	0.48	0.20	310	17.0
3	0.14	0.09	60				18.0
4	0.13	0.08	114				19.0
5	0.11	0.05	100	0.53	0.27	621	18.0
6	0.15	0.06	116				19.5
7	0.11	0.04	59				18.0
8	0.15	0.05	116	0.53	0.26	360	18.5
Density 8							
1	—	—	—				17.0
2	0.17	0.08	59	0.47	0.23	325	17.0
3	0.17	0.08	60				18.0
4	0.18	0.08	50				19.0
5	0.12	0.06	114	0.58	0.31	382	18.0
6	0.17	0.09	118				19.5
7	0.13	0.04	59				18.0
8	0.18	0.05	52	0.60	0.28	638	18.5

Note: Water temperature is the temperature at a depth that corresponds to the center of the enclosures.

enclosures characterized by similar environmental conditions (fish density, water temperature, and zooplankton characteristics) were 38.2 and 42.6 cal·h^{-1}. We found no significant difference between these values ($F_{1,7} = 0.757$, $p > 0.40$). Furthermore, there was no significant difference between hourly activity rates of fish that had spent 3 or 27 days inside our enclosures (average difference = 8%; $F_{1,7} = 0.888$, $p > 0.35$). During our study, the proportion of the enclosure that was under observation during filming ranged from 4 to 40% because of variations in visibility. However, we found no relationship between daily activity rates and the proportion of the enclosure filmed ($F_{1,16} = 0.14$, $p > 0.71$). Hence, our estimates of activity were not influenced by the proportion of the enclosure under observation. We also interpret that whether or not we sampled the corner of our enclosures (observed only during high visibility) had no effect on our activity rates. Our tests, together with those performed by Boisclair (1992), Boisclair and Sirois (1993), and Sirois and Boisclair (1995), support the assumptions we adopted to execute our sampling and analyses.

Energetic consequences of differences in fish density

Our experiments allowed us to assess the mechanism by which an increase in fish density influenced fish growth. As expected, growth increment of individuals inside the enclosure containing four fish was larger than that estimated for fish held at twice that density (individual growth difference = 254 cal) (Table 2). Furthermore, higher growth was associated with a marginally higher consumption rate (difference in energy intake = 103 cal). In this sense, our work provided little support for the hypothesis that food base limitation contributed to a decrease in growth rate because, in our study, differences in consumption rates of fish at two experimental densities were not statistically significant. However, the difference in feeding rate explained 40% of the growth difference between fish held at the two experimental densities (Table 2). Our work also indicated that fish held at a density of four fish per enclosure spent less energy swimming than those at the higher density (activity difference = 156 cal). While our data supported the hypothesis of the influence of behavioral interactions on fish growth, they also suggested that this mechanism explained

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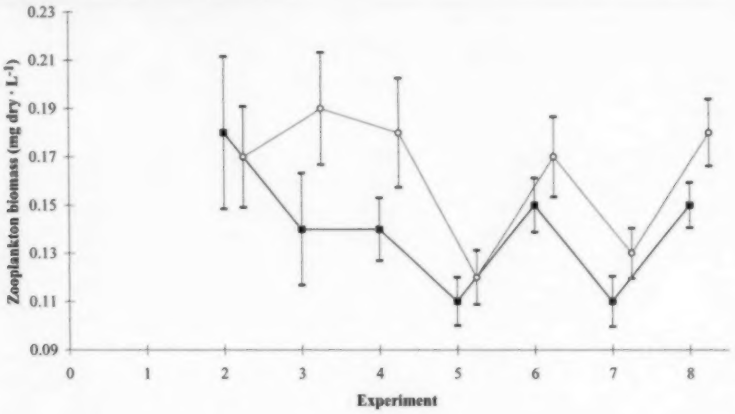
Fig. 2. Mean zooplankton biomass inside the enclosures containing four (D4 enclosures, solid squares) or eight brook trout (D8 enclosures, open circles). Vertical bars represent 95% confidence intervals.



60% of the growth difference we observed between the two experimental densities. Our analysis further indicated that other energy expenditures (OEE) estimated by difference (OEE = consumption – growth – activity) were similar and represented 37% (295 cal) of the energy budget of fish in the D4 enclosures and 42% (290 cal) of that of fish in the D8 enclosures.

Discussion

Our results support the long-standing hypothesis that fish density has a negative effect on fish growth rates (Boisclair and Leggett 1989*a*; Jørgensen et al. 1993; Mazur et al. 1993). The most common explanation for this inverse relationship is that as fish density increases, the relative abundance of prey, the quantity of food consumed by individual fish, and consequently the growth rates of fish decrease (Henderson 1985; Henderson and Brown 1985). However, nonexploitative interactions have also been argued to contribute to the existence of a negative relationship between fish growth and density (Jobling 1985; Boisclair and Leggett 1989*c*). This mechanism presumes that as fish density increases, the number of interactions among fish (hampering, aggressions, chases, intimidation) increases and has a negative effect on fish foraging efficiency and more specifically on the costs of acquiring a given ration level (Jobling 1985; Jobling and Reinses 1986). Our analyses suggest that, under the experimental conditions found in our enclosures, a doubling in fish density had no statistically significant effect on consumption rates but resulted in a significant increase in fish activity rates. Considering the error around our estimates of growth, consumption, and activity rates, we infer that the decrease in feeding rates and the increase in activity rates explained a similar fraction of the growth differences observed between our two experimental densities. Hence, in our study, both exploitative and nonexploitative interactions appear to have played a similar role.

The relevance of our results and interpretations for field situations is difficult to evaluate but can be expected to depend on the difference between the conditions found in our enclosures and those that occur in lakes. The most important difference between our enclosures and a lake may be the fish density we used. Our study was realized with planktivorous fish held at 0.5–1 fish·m^{-3}. Traditional approaches to estimate fish abundance or density such as mark–recapture experiments and trawling generally average out fish abundance over large areas or volumes (Beverton and Holt 1957). Such estimates of fish density often range from 0.1 to 4 fish·m^{-2}. However, hydroacoustic surveys indicate that estimates of fish density may vary 10-fold within the length of the transects and within the volume of water sampled, mostly because of the spatial heterogeneity of fish distribution (Luecke and Wurtsbaugh 1993; Molls and Neumann 1994). The spatial resolution provided by hydroacoustic systems indicates that local fish densities similar to and even above those used in our experiments may in fact be rather common (Brabrand and Faafeng 1993; Molls and Neumann 1994).

Another potentially important difference from natural conditions may be that fish density was artificially held constant by our enclosures. This contrasts with natural conditions in which fish density is known to vary at temporal scales ranging from minutes to hours (Brabrand and Faafeng 1993; Helfman 1993). For instance, fish schools have been known to break and reconstruct under the influence of the presence of predators (Pitcher and Parrish 1993). Fish schools may also form during specific periods of the day and dissolve into relatively homogenous concentrations at other times (Luecke and Wurtsbaugh 1993). Our experimental approach did not allow fish to modify their densities to an extent similar to that found under natural conditions.

One implication of our work may be that the relative importance of exploitative and nonexploitative interactions may partly depend on the proportion of time fish spend at various densities. However, it is also important to recognize that prey abundance may affect fish distribution and behavior, their growth, consumption, and activity rates, and consequently the relative effect of exploitative and nonexploitative interactions. During our study, fish at the two experimental densities were held at similar levels of prey abundance. The suitability of this condition to represent field situations depends on the expected relationship between the abundance of fish and that of their prey. Although there are, to our knowledge, no published accounts of such relationships, many studies in which abundance

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and biomass units have been used for either variable may be taken as indications of the potential relationship between fish and their prey. Manipulations performed to assess the effect of fish abundance on their prey resource suggest that there should be a negative relationship between fish biomass and prey abundance (Larkin 1978; Carpenter et al. 1985). However, such observations are generally performed during relatively short time intervals, and it is difficult to assess if high fish abundance could be maintained on low prey abundances for longer periods. In contrast, empirical analyses that relate fish and prey abundance or basic production (such as total phosphorus) collected from a range of environmental conditions support the existence of a positive relationship between these variables (Vanni and Findlay 1990). However, these empirical analyses also reveal substantial variation around any relationship relating the abundance or biomass of fish to that of their prey. The magnitude of this variation suggests that important differences in the abundance of fish for a given prey abundance can be expected, and consequently that our experiment may represent a subset of realistic environmental conditions.

Many studies have been performed to describe and explain patterns of energy partitioning in fish. For instance, Kerr (1982) used published values of fish growth and consumption rates to develop a model suggesting the existence of a positive relationship between activity and feeding rates. This model was supported by the data of Boisclair and Leggett (1989*a*, 1989*b*) and Madon and Culver (1993), but, in all cases, feeding and activity rates were not statistically independent because activity was estimated by difference using values of growth and consumption rates. In contrast, Post and McQueen (1994) proposed that there had to be a negative relationship between consumption rates and activity rates or prey abundance. They based their argument on the observation that published values of 0+ yellow perch growth rates vary by an order of magnitude and on their interpretation that such variability is not consistent with the relationship proposed by Kerr (1982) which has a tendency to stabilize fish growth efficiency. Neither consumption nor activity rates were estimated by Post and McQueen (1994).

Studies aimed at obtaining independent estimates of fish growth, consumption, and activity rates under different environmental conditions could be expected to allow the discrimination of contrary predictions. However, examination of our data, together with published independent estimates of feeding and activity rates, results in apparently conflicting interpretations. For instance, the studies of Boisclair (1992), Boisclair and Sirois (1993), and Sirois and Boisclair (1995) in which consumption and activity rates were estimated independently support the existence of a positive relationship between feeding and activity rates. In contrast, our data tend to agree with a negative relationship between feeding and activity rates (fish in the D8 enclosures had lower feeding rates but higher activity rates than fish in the D4 enclosures). It is interesting to note that in the studies that supported the existence of a positive relationship between feeding and activity rates, these variables were modified by changing prey availability for a given fish abundance (or as in the data used by Kerr (1982), more food was given to the fish). The only study that tended to support a negative relationship between these variables involved the manipulation of fish abundance for a given prey abundance. Taken together, these apparently contradictory studies can be reconciled by widening the model proposed by Kerr (1982), i.e., by proposing that both feeding and activity rates may increase with prey abundance for a given fish density (as supported by Boisclair and Sirois (1993), Madon and Culver (1993), and Sirois and Boisclair (1995)) and that feeding may decrease and activity may increase as fish density increases for a given prey abundance (as suggested by Post and McQueen (1994) and supported by our study).

Because most studies test one independent variable at a time and because the range of environmental conditions examined is still narrow, it is difficult at present to thoroughly evaluate the relative importance of prey abundance and fish density to fish energy acquisition and partitioning. However, as a preliminary analysis, we combined the data obtained in this study with those published by Boisclair and Sirois (1993) and Sirois and Boisclair (1995) to assess the validity of our view that both prey and fish abundance (or biomass) may affect fish growth, consumption, and activity rates. All data used during this analysis focused on a single species, the brook trout, but fish weight ranged from 0.4 to 3.5 g wet. To avoid problems associated with the pooling of different fish sizes in a single analysis, we scaled all bioenergetic components as multiples of fish standard metabolic rate (e.g., activity was defined as activity rate/standard metabolic rate). The data set contained 30 series of independent estimates of growth, consumption, activity, and associated prey biomass (grams dry per litre), fish abundance (number per cubic metre), and biomass (grams wet per cubic metre). Fish biomass was the only variable that explained a significant fraction of growth variability ($F_{1,30} = 331.16$, $p < 0.0001$, $r^2 = 0.92$). Fish biomass ($F_{1,30} = 23.04$, $p < 0.0001$, partial $R^2 = 0.54$) and zooplankton biomass ($F_{1,30} = 4.25$, $p < 0.05$, partial $R^2 = 0.07$) explained a total of 61% of the variability of consumption rates. Consumption tended to decrease with an increase in fish biomass, but consumption increased with an increase in zooplankton biomass. Finally, we found a significant relationship ($R^2 = 0.35$) between fish activity and fish biomass ($F_{1,30} = 12.7$, $p < 0.0015$, partial $R^2 = 0.18$) and zooplankton biomass ($F_{1,30} = 7.15$, $p < 0.015$, partial $R^2 = 0.17$). Activity tended to increase with an increase in both fish and prey biomass. These analyses support our suggestion that feeding and activity rates may be affected by both fish and prey biomass. In addition, these relationships are consistent with our proposal that both feeding and activity rates may increase with prey abundance and that feeding may decrease and activity may increase as fish density increases.

We recognize that we did not replicate our experiments and that this represents a weakness in our work. This lack of formal replication is related to logistical limits (the number of individuals required to handle and maintain the enclosures and the time needed to analyse a large number of films within budgetary constraints) and not to repeatability problems. Within this context, there appear to be three approaches available to contribute to the study of interactions between fish growth, consumption, and activity rates: (*i*) develop models using direct estimates of growth and consumption rates and indirect estimates of activity rates where activity values are not statistically independent of the two other major components (Kerr 1982; Boisclair and Leggett 1989*c*), (*ii*) develop models using other models and no estimate of activity and consumption rates (Post and McQueen 1994), and (*iii*) develop models by slowly gathering independent estimates of growth, consumption, and

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activity rates through a series of experiments that, taken individually, are repeatable but logistically too laborious to replicate (Boisclair 1992; Boisclair and Sirois 1993; Sirois and Boisclair 1995; this study). We selected the third approach with the contention that it currently represents the only alternative that may, in the long run and with the patience of our colleagues rightly concerned with statistical designs, allow development of empirical models based on biologically realistic estimates of key components of bioenergetic models. At this point, the criteria we used to define "biologically realistic" data are admittedly imperfect: Does the energy budget determined within one experiment balance when consumption and activity rates estimated for one enclosure are combined with values of consumption rates obtained for five other enclosures? Are there discernable patterns that emerge among a series of independent experiments? Are these patterns consistent or reconcilable with expectations based on other approaches? The meeting of these criteria, together with the support provided by all attempts to verify the validity of the assumptions underlying our approach, suggests that the strategy we adopted may provide coherent and interpretable data.

Our results contrast with the basic assumption of fish bioenergetic models that fish density influences fish growth mostly, if not only, through its influence on consumption rates. One potential implication of this situation is that attempts to estimate, for instance, fish feeding rates with bioenergetic models that do not account for the influence of fish or prey density on fish energy expenditures or activity rates could be biased. This point can be illustrated using our own data. We implemented the computer version of a bioenergetic model proposed by Hewett and Johnson (1992) with our values of water temperature, fish weight, prey taxonomic composition, and caloric density (fish and zooplankton). We simulated the growth of fish in the D4 and D8 enclosures separately using the model parameters of sockeye salmon (*Oncorhynchus nerka*). We assumed that activity costs of fish in both densities were identical (0.4 times standard metabolic rate). Total consumption predicted by the bioenergetic model was within 5% of our observed value for fish in the D4 enclosures but 250% lower than our values for fish in the D8 enclosures. While the work required to explore energy partitioning in fish may be laborious, our study suggests that such studies may be necessary to improve the accuracy associated with the predictions of bioenergetic models.

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Trade-offs between herbivore resistance and competitiveness in periphyton of acidified lakes

Mark D. Graham and Rolf D. Vinebrooke

Abstract: It was hypothesized that herbivores regulate periphyton succession in acidified lakes by suppressing loosely attached filamentous algae that otherwise persist and outcompete other growth forms for resources, such as dissolved inorganic carbon (DIC). This hypothesis was tested by conducting grazer-exclosure experiments over an ice-free season in acidified and circumneutral lakes that differed in DIC concentrations. In each lake, the two-factor experimental design had two herbivore (presence–absence) and five succession (early–late) treatments. Total periphyton biomass was greater and significantly suppressed by herbivores in the circumneutral lake. Here, acid-sensitive green frog (*Rana clamitans*) tadpoles suppressed loosely attached (i.e., zygnematacean taxa) while stimulating firmly attached (i.e., *Coleochaete scutata*) algae, causing succession towards a herbivore-resistant physiognomy. Conversely, a loosely attached and tall physiognomy persisted in the absence of significant grazing and greater resource limitation in the acid lake. Our findings suggest that trade-offs between competitiveness and herbivore resistance help predict changes in periphyton in recovering acidified lakes. During recovery, concomitant increases in herbivory and resources should favour a firmly attached and short periphyton physiognomy that is resistant to removal by herbivores, but that lacks the competitiveness of loosely attached and tall forms in DIC-limited acid lakes.

Résumé : On a émis l'hypothèse que les herbivores régulent la succession du périphyton (pris ici au sens de la microflore épibionte) dans les lacs acidifiés en supprimant les algues filamenteuses faiblement fixées qui autrement persisteraient et domineraient les autres formes de vie dans la compétition pour les ressources, notamment pour le carbone inorganique dissous (CID). On a testé cette hypothèse en effectuant des expériences d'exclusion des brouteurs durant toute une saison d'eau libre de glace dans des lacs acidifiés et presque neutres ayant des concentrations de CID différentes. Dans chaque lac, le protocole expérimental à deux facteurs comportait deux traitements pour ce qui est des herbivores (présence–absence) et cinq traitements pour ce qui est de la succession (jeune–avancée). La biomasse totale de périphyton était supérieure et supprimée de façon significative par les herbivores dans le lac presque neutre. Dans ce lac, les têtards de grenouille verte (*Rana clamitans*) sensibles à l'acidité ont supprimé les algues faiblement fixées (c.-à.-d., zygnématacées) et ont stimulé le développement des algues fermement fixées (c.-à.-d., *Coleochaete scutata*), faisant ainsi évoluer la succession vers une physionomie caractérisée par des végétaux résistants aux herbivores. Réciproquement, une physionomie caractérisée par des algues faiblement fixées et de grande taille a persisté en l'absence d'un broutage significatif et avec une plus grande limitation des ressources dans le lac acide. Nos résultats laissent penser que la relation entre la compétitivité et la résistance aux herbivores aide à prévoir les changements dans le périphyton dans les lacs acidifiés en voie de rétablissement. Durant le rétablissement, des accroissements concomitants des populations d'herbivores et des ressources devraient favoriser le développement d'une physionomie caractérisée par la présence d'un peuplement végétal fermement fixé et court, résistant à l'attaque des herbivores, mais qui n'est pas aussi compétitif que les formes allongées faiblement fixées dans les lacs acides renfermant de faibles concentrations de CID.
[Traduit par la Rédaction]

Introduction

Algal physiognomy is being increasingly recognized as an important determinant of how periphyton responds to abiotic and biotic factors, such as herbivory (Rosemond 1996; Steinman 1996) and resource competition (McCormick 1996). For example, periphyton can become increasingly resistant to herbivory during succession towards a firmly attached or prostrate structural organization (Rosemond 1996). Conversely, a tall and filamentous periphyton physiognomy may enhance overall uptake of certain resources, such as limiting nutrients (McCormick 1996).

The proliferation of filamentous zygnematacean algae commonly occurs in the periphyton of acidifying lakes, suppressing an understory of shorter and smaller cyanobacteria and diatoms (Stokes 1986; Vinebrooke 1996). Loosely attached, filamentous growth forms are viewed as being superior competitors (sensu Tilman 1982) for dissolved inorganic carbon (DIC), a resource that increasingly limits periphyton during lake acidification (Fairchild and Sherman 1992; Turner et al. 1995). Their tall stature may allow them to extend up into the upper boundary layer where DIC availability is higher due to water flow enhancing gas diffusion. By forming an overstory, loosely attached filamentous forms may as a consequence outcompete adnate and prostrate forms for other resources, such as light and inorganic nutrients (McCormick 1996).

Herbivory is also considered as an important regulator of

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periphyton in acidified lakes (Stokes 1986). Declines in herbivore abundances have been associated with increases in the abundance of filamentous green algae in acidified lakes (Appelberg et al. 1993). In both acidified lakes and streams, periphyton taxa typically show differential sensitivities to grazing (Planas 1996). Herbivores can remove loosely attached taxa and stimulate growth by herbivore-resistant small and adnate or large and prostrate taxa under conditions of low acidity (pH ~ 6) (Vinebrooke 1996). Therefore, functional responses, based on changes in overall community physiognomy, may help explain changes in periphyton biomass and species composition during lake acidification.

Trade-offs between competitiveness and herbivore resistance in algae may be demonstrated over a successional timescale (Tuchman and Stevenson 1991; McCormick 1996). For example, after ice-out, spring–summer lake succession can result in the replacement of edible early successional species by later successional species that are relatively resistant to herbivores (Power et al.1996). If such trade-offs exist in the case of loosely attached filamentous periphyton in acidifying lakes, then they should represent a functional group with predictable behaviours. Functional form theory (Rosemond 1996) might predict that a loosely attached, tall periphyton physiognomy is favoured by severe resource limitation and will persist in the absence of significant grazing pressure. We tested this hypothesis by conducting an experiment that examined the development of periphyton in the presence and absence of herbivores in acidified lakes that differed in their degree of DIC limitation.

Methods

Study lakes

The experiment was conducted in Little Sheguiandah Lake and Ruth-Roy Lake, two small (<46.1 ha) oligotrophic lakes located within Killarney Provincial Park (46°02′N, 81°24′W), Ontario, Canada. Little Sheguiandah Lake has a predominantly granitic basin, while Ruth-Roy Lake is situated on a mixed granitic and quartzite basin. These lakes have shown partial chemical recovery from anthropogenic acidification due to reductions in acidic deposition from nearby (~80 km) Sudbury smelters (see Vinebrooke and Graham 1997). Little Sheguiandah Lake is a circumneutral and marginally mesotrophic lake, while Ruth-Roy Lake has remained highly acidic and oligotrophic. Little Sheguiandah Lake contains higher abundances of large herbivores, such as green frog (*Rana clamitans*) tadpoles and cyprinids, while the zoobenthos in Ruth-Roy consists mainly of crayfish (*Cambarus robustus*, *Cambarus bartoni*), anisopteran odonates, and chironomids.

Water chemistry analyses

Water samples were taken monthly from the outflow of each lake. Lake water pH and conductivity were measured in the field using a Corning model 610 portable pH meter and a Fisher model 152 conductivity meter. Unfiltered water samples were measured for DIC using total carbon infrared gas analysis. Filtered (0.45-µm Nucleopore) water samples were preserved with 1% HNO_3 and analyzed for phosphorus concentrations using inductively coupled plasma mass spectrometry (Ontario Ministry of the Environment 1981).

Invertebrate sampling and enumeration

Grazers in each lake were sampled monthly using a Surber sampler and visual surveys along the shallow littoral area. The Surber sampler was modified by affixing a foam collar to the brass frame so as to create a better seal with the benthic substrate and placing a plastic sleeve over the opening of the sampler to prevent the loss of highly mobile individuals. Three 5-m-long transects that each ran parallel to the shore were surveyed from canoe to estimate the densities of large grazers that were not easily sampled with the surber sampler (e.g., cyprinids). Taxonomic references included Merritt and Cummins (1984).

Experimental design

We used separate two-factor experiments to test for the effects of herbivores and successional development on biomass accrual by periphyton and defined physiognomic groups in the acid and circumneutral lakes. In each lake, there were 10 treatment combinations (two herbivore levels × five developmental stages) replicated three times for a total of 30 unglazed square tiles (100 cm^2). Three sets of 10 tiles were each randomly placed in both lakes along rock ledges at a depth of 0.5 m in early May 1992. Grazers were excluded from half of the substrates using individual 5-mm-mesh cages (10 × 10 × 12 cm), each containing a single tile. The successional development of periphyton was determined by monthly sampling of randomly selected tiles from June to October. Although the acid and circumneutral lake conditions were not replicated, differences between severely and moderately DIC-limited periphyton responses could be detected, but not attributed to treatment.

Periphyton sampling and enumeration

Tiles were retrieved by placing a plastic container over each tile prior to its removal from the lake to minimize loss of loosely attached algae. Algae were harvested with a razor scraper and coarse toothbrush and preserved with Lugol's. Samples were homogenized and sonicated before sedimentation in Utermöhl chambers. Most algae were identified and counted at a magnification of 400×. However, large colonial and filamentous algae were separately counted at 250× to improve the accuracy of density estimates for large taxa. Cell viability was based on chloroplast integrity. A minimum of 100 cells of the dominant taxon and at least 300 cells in total were counted. Algal counts from three replicated subsamples were converted to biovolumes by approximating cell shapes with geometric forms of known volumetric formulae (Wetzel and Likens 1991). Algal samples were identified using Patrick and Reimer (1966, 1975) for diatoms and Prescott (1982) for all others.

Four physiognomic groups of periphyton were each identified based on a set of common morphological features shared by the different taxa (Table 1). A firmly attached, tall group comprised erect and filamentous taxa that had conspicuous attachment structures (i.e., basal holdfast cell or stalked attachment). Examples included oedogoniacean taxa with specialized basal holdfast cells and long-stalked diatoms, such as *Cymbella* and *Gomphonema*. Adnate and prostrate taxa that had the potential for being strongly affixed to surfaces were assigned to a firmly attached, short group. Short-stalked diatoms (e.g., *Achnanthes*) and prostrate green colonials (e.g., *Coleochaete*, *Stigeoclonium*) would be included in this group. Taxa that lacked obvious attachment structures and instead had weakly adhering capabilities (e.g., mucilaginous excretions) were assigned to either short or tall, loosely attached groups based on their respective statures (i.e., unicellular or small colonial versus long and filamentous). Unicellular desmids and colonial cyanobacteria were considered loosely attached and short, while filamentous zygnematacean taxa are examples of loosely attached, tall forms.

Statistical analyses

Total periphyton and physiognomic group biovolume accruals were separately analyzed by two-factor (herbivory, time) analysis of variance (ANOVA) (SYSTAT version 6, SYSTAT Inc., Evanston, Ill.). Repeated-measures ANOVA was not used in this study, as physically separate tiles were randomly sampled each month (Scheiner and Gurevitch 1993). Algal biovolumes were log transformed to stabilize

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Table 1. Classification of sampled epilithic algal taxa into four defined physiognomic groups based on attachment mechanism and stature.

Loosely attached, tall	Firmly attached, tall	Loosely attached, short	Firmly attached, short
Groenbladia undulata	*Bulbochaete* spp.	*Actinotaenium cucurbitinum*	*Achnanthes minutissima*
Klebsormidium sp.	*Oedogonium* spp.	*Chroococcus* spp.	*Coleochaetae scutata*
Mougeotia spp.	*Cymbella cuspidata*	*Closterium* spp.	*Homeothrix juliana*
Spirogyra spp.	*C. lunata*	*Cosmarium* spp.	*Synedra* sp.
Spondylosum moniliforme	*C. minuta*	*Cylindrocystis brebissonii*	
Zygnema sp.	*C. subcuspidata*	*Euastrum* spp.	
Zygogonium spp.	*Gomphonema acuminatum*	*Eunotia* spp.	
	G. truncatum	*Eunotia exigua*	
		Frustrulia rhomboides	
		Gonatozygon spp.	
		Merismopedia spp.	
		Navicula spp.	
		Navicula subtilissima	
		Stauroneis spp.	
		Tabellaria fenestrata	
		T. flocculosa	

variance and approximate normal variance distributions prior to statistical analyses. Tukey's tests with Bonferroni adjustment were used for a posteriori multiple comparisons.

The rate of periphyton succession was determined by regression analyses of monthly changes in the community similarity index with time (Tuchman and Stevenson 1991). Similarity index values were based on the biovolumes of the four physiognomic periphyton groups.

Results

Nutrient concentrations and herbivore abundances

Nutrient concentrations differed between the two lakes throughout the entire ice-free season (Table 2). DIC concentrations were consistently over threefold times higher in the circumneutral lake, showing a progressive decline throughout the ice-free season. Similarly, pH declined in the circumneutral lake, but remained >1.5 units above that recorded in the acid lake. Total phosphorus concentrations were only slightly higher in the circumneutral lake than in the acid lake.

The circumneutral lake contained a higher diversity and abundance of herbivores that were excluded from periphyton contained within the exclosures (Fig. 1). Here, overlapping cohorts of green frog tadpoles caused tadpoles to be the dominant benthic grazer. Crayfish (*Cambarus robustus*, *Cambarus bartoni*) were most abundant in the later months, reaching densities that were comparable with those found in the acid lake. Crayfish were the only large grazers in the acid lake and were at densities that remained relatively constant throughout the ice-free season.

Periphyton biomass

Herbivores significantly suppressed total periphyton biovolume in the circumneutral lake (ANOVA; $F_{1,20} = 7.78$, $P = 0.01$) but not in the acid lake ($F_{1,20} = 0.41$, $P = 0.53$) (Fig. 2). A significant herbivore–time interaction ($F_{4,20} = 4.31$, $P = 0.01$) affected periphyton in the circumneutral lake as a result of a temporal shift from a net negative to a net positive effect of herbivory during August–September (Fig. 2). Herbivore stimulation of periphyton development in the circumneutral lake followed the period of maximum tadpole abundance in August (Fig. 1).

Table 2. Select water chemistries of the two experimental lakes based on monthly sampling of lake outflows.

Lake	Month	pH	DIC (mg/L)	P (µg/L)
Ruth-Roy	June	4.15	0.06	6.7
	July	4.20	0.07	7.0
	August	4.10	0.05	7.8
	September	4.55	0.09	7.6
	October	4.00	0.05	7.8
Little Sheguiandah	June	6.80	1.50	7.6
	July	6.45	1.30	8.2
	August	6.45	1.30	10.8
	September	6.30	1.00	9.6
	October	6.05	0.70	8.2

Note: DIC, dissolved inorganic carbon; P, total dissolved (0.45-µm filterable) phosphorus.

Total periphyton biovolume accrual significantly peaked during late summer in the circumneutral lake (time effect; $F_{4,20} = 4.51$, $P = 0.009$), but did not show a clear seasonal pattern on tiles in the acid lake ($F_{4,20} = 1.96$, $P = 0.14$) (Fig. 2). Periphyton biovolume in the circumneutral lake was lowest in August, concomitant with the period of maximum herbivore activity (Figs. 1 and 2). Also, total periphyton abundance was about two times greater in the circumneutral lake than in the acid lake during the entire season (Fig. 2).

Periphyton physiognomy

The overall structural organization of periphyton communities on the open control tiles differed between the two lakes (Fig. 3). Firmly attached, short growth forms succeeded loosely attached taxa as the dominant group by late summer in the circumneutral lake, while periphyton consisted primarily of loosely attached growth forms throughout the entire ice-free season in the acid lake. The subsequent rapid decline of the firmly attached, short group by October in the circumneutral

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Fig. 1. Mean ($n = 3$) densities of excluded (>5 mm size) grazers in the circumneutral and acid lakes during the 1992 ice-free season.

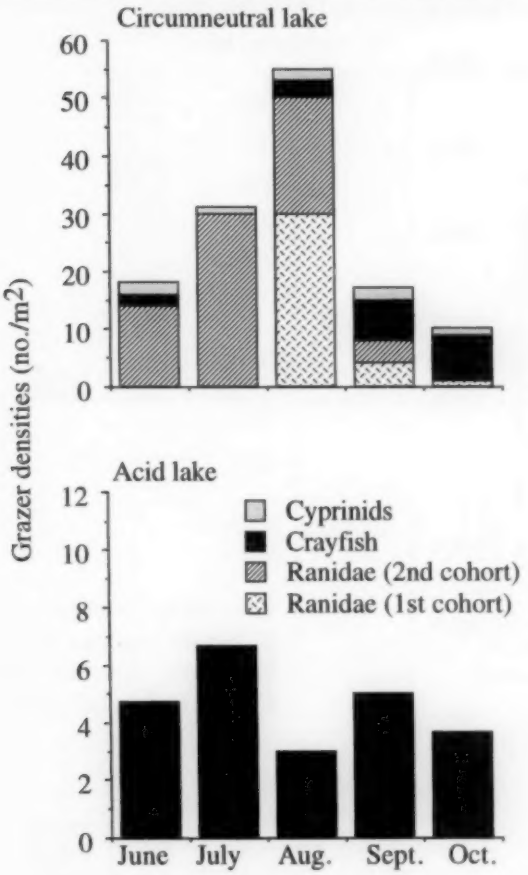
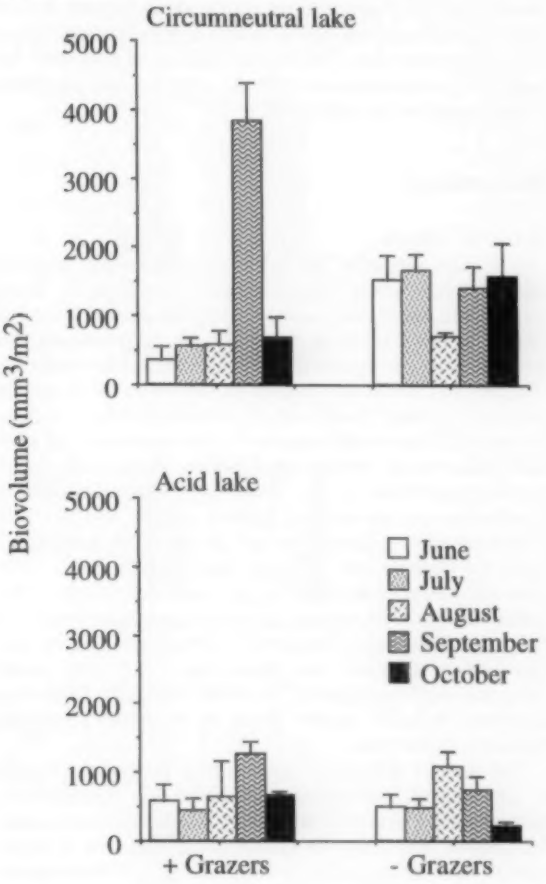


Fig. 2. Mean (±1 SE) total periphyton biovolume accrual on tiles from treatments with (+ grazers) or without (– grazers) exposure to large grazers in the circumneutral and acid lakes during the 1992 ice-free season.



lake was due to the senescence of a prostrate thallus-forming green alga (*Coleochaete scutata*; see Table 1).

Loosely attached, tall periphyton biomass was greater in the acid lake than in the circumneutral lake (Fig. 4), as filamentous zynematacean genera (*Mougeotia*, *Zygogonium*) were 50% more abundant under relatively low-resource conditions. In the acid lake, the abundance of this physiognomic group was unaffected by herbivory (Table 3) and significantly increased over the first half of the growing season (time effect; Fig. 4). In contrast, herbivory in the circumneutral lake had a significant net negative effect on loosely attached, tall periphyton (Table 3). Further, time significantly favoured the development of this growth form in the circumneutral lake (Fig. 4).

Loosely attached, short periphyton biomass was lower in the acid lake, as many unicellular desmids (i.e., *Closterium*, *Cosmarium*) and other greens (*Pediastrum*) were more abundant in the circumneutral lake (Fig. 4). This physiognomic group was unaffected by herbivory and time in the acid lake (Table 3). However, herbivory had a highly significant negative effect on loosely attached, short growth forms in the circumneutral lake (Fig. 4). Time had a marginally significant effect on this group, as its abundance declined with the development of periphyton.

Firmly attached, tall periphyton was more abundant in the circumneutral lake, as stalked diatoms and filamentous greens with basal holdfasts were 75% less predominant under conditions of severe resource limitation in the acid lake (Fig. 4). In the circumneutral lake, herbivory had a net positive effect on biomass accrual by firmly attached, tall growth forms (Table 3; Fig. 4). This group was unaffected by herbivores and time in the acid lake.

Firmly attached, short growth forms were more abundant in the circumneutral lake than in the acid lake, where they constituted <5% of the total periphyton biomass (Fig. 4). Herbivores had a highly significant positive effect on firmly attached, short periphyton in the circumneutral lake (Table 3; Fig. 4). A significant herbivore–time interaction was due to the stimulatory effect of grazing occurring primarily in September. The abundance of firmly attached, short periphyton significantly increased over the summer and thereafter abruptly declined (time effect; Fig. 4). Neither herbivores nor time significantly affected the abundance of firmly attached, short periphyton in the acid lake.

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Periphyton succession

Periphyton succession was promoted by herbivory in the circumneutral lake (Fig. 5). Here, succession in grazed periphyton consisted of firmly attached growth forms succeeding loosely attached forms over the course of the summer. In contrast, successional rate was slower in ungrazed periphyton in the circumneutral lake. Regression analyses also showed that periphyton succession was both not apparent and unaffected by herbivores in the acid lake (Fig. 5).

Discussion

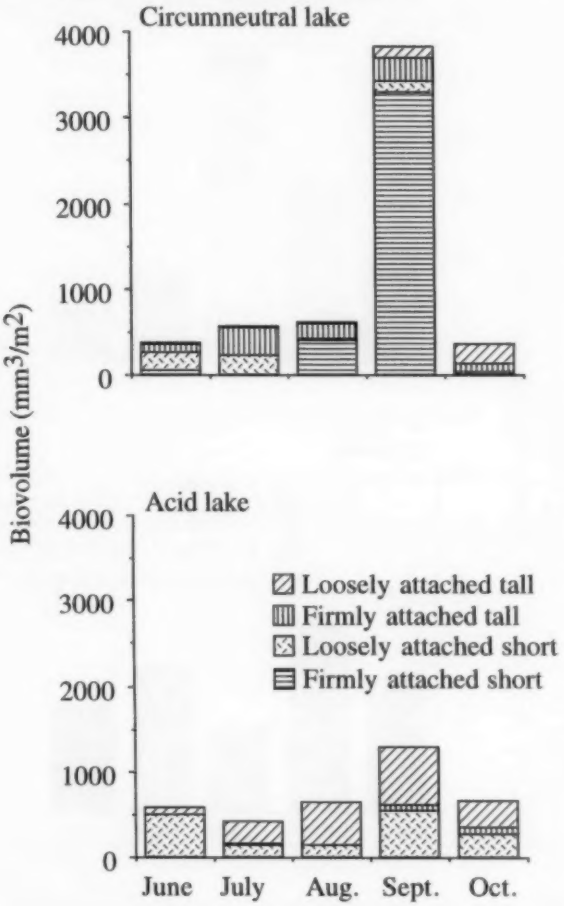
Herbivore effects

Our findings supported the hypothesis that loosely attached, tall periphyton would persist throughout the growing season under conditions of low grazing activity that existed in the acid lake or within grazing exclosures in the circumneutral lake (Fig. 4). Also, we showed that the net effect of herbivores on total periphyton abundance could be viewed as the sum of the differing responses by defined physiognomic groups of algae. For instance, the overall impact of herbivores on total periphyton biomass in the circumneutral lake varied due to the differential susceptibilities of the various physiognomic groups to disturbance and ingestion by grazers (Figs. 4 and 5). Here, grazers suppressed loosely attached growth forms while stimulating the development of firmly attached periphyton. Thus, gains and losses by the different growth forms may have prevented the complete removal of periphyton by herbivores in the circumneutral lake. Increases in nutrient recycling by benthic grazing (Osborne and McLachlan 1985; McCormick 1996) and inefficient grazing pressures (Steinman 1996) may also have mediated against larger net losses of periphyton biomass to herbivores.

The basis for differential resistances to herbivores amongst the physiognomic groups appeared to involve differences in their respective modes of attachment. Basal attachment, rather than stature, was likely the primary determinant of susceptibility to removal by grazers in this study. Firmly attached taxa were most resistant to removal, which likely involved physical disturbance, in additon to ingestion, by the large herbivores. In fact, nonconsumptive physical disturbance of periphyton by herbivores can account for a large fraction of their overall impact and result in the overestimation of actual grazing rates that are attributable to direct ingestion (Cattaneo and Mousseau 1995). The positive net effect of herbivory on both firmly attached, short *Achnanthes minutissima* and *Coleochaete scutata* and tall oedogoniacean taxa has also been reported in nonacidified systems for these (Cattaneo and Kalff 1986) and other firmly attached, short, or filamentous algae, such as *Cladophora*. (Steinman 1996). The absence of *Coleochaete scutata* on ungrazed tiles suggested that this species was "grazer dependent", as is the basal form of *Stigeoclonium* in some streams (Rosemond 1996). Although stature is commonly emphasized (see review in Steinman 1996), our findings suggest that basal attachment can be of equal or greater importance in determining herbivore resistance in periphyton.

Significant herbivore–succession interactions showed that the net effects of herbivory on periphyton physiognomy changed over the growing season. There was a shift from a net negative to a net positive effect of herbivory on total

Fig. 3. Mean ($n = 3$) physiognomic composition and total biovolume of periphyton on open control tiles in the circumneutral and acid lakes during the 1992 ice-free season.



periphyton biomass in the circumneutral lake. This shift corresponded to an increasing dominance by a firmly attached, prostrate green alga *Coleochaete scutata*. *Coleochaete scutata* showed a slow biomass accrual rate but eventually replaced earlier successional forms that were predominantly loosely attached and, as a consequence, easily disturbed and ingested by tadpoles. In other tadpole-dominated systems, periphyton biomass can be substantially reduced due to the removal of loosely attached filamentous algae (Dickman 1968), especially during early succession (Lamberti et al. 1992). In comparison, we showed that the long-term (4 months) temporal shift from a net negative to positive effect of herbivory could be attributed to the development of an increasingly herbivore-resistant algal physiognomy that came to dominate the periphyton during the most intensive grazing period in the circumneutral lake. Similar successional patterns of increasing herbivore resistance have been reported for phytoplankton and lotic periphyton (see Power et al. 1996).

Herbivory promoted periphyton succession only within the circumneutral lake (Fig. 5). Here, succession occurred more quickly on grazed tiles as herbivore-resistant, firmly attached

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Fig. 4. Mean (±1 SE) biovolume accrual by loosely attached and firmly attached periphyton of tall and short stature on tiles from treatments with (+ grazer) or without (– grazers) exposure to large grazers in the circumneutral and acid lakes during the 1992 ice-free season.

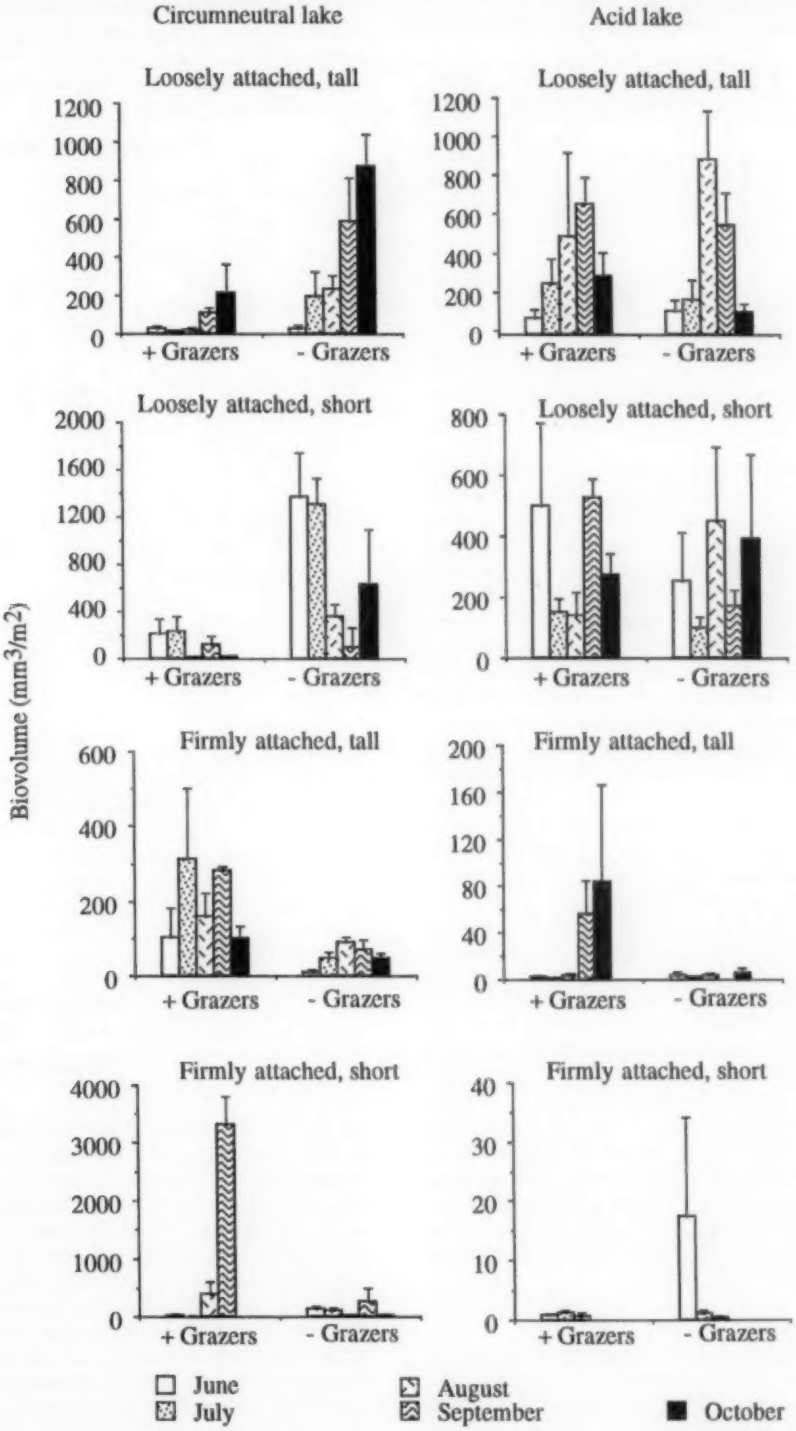

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Table 3. ANOVA results for the effects of herbivores and succession on biovolume accrual by different periphyton physiognomies in the circumneutral and acid lakes.

		F			
Treatment	df	Loose and tall	Loose and short	Firm and tall	Firm and short
Circumneutral lake					
Herbivores	1	18.18***	26.48***	9.94**	30.40***
Time	4	6.99***	3.13*	1.32	34.17***
Herbivores × time	4	2.60	1.52	0.91	27.50***
Acid lake					
Herbivores	1	0.01	0.21	2.33	0.89
Time	4	4.27*	0.78	1.00	1.07
Herbivores × time	4	0.76	1.39	0.95	0.98

Note: Physiognomic groups were based on mode of attachment (loose versus firm) and relative stature (tall versus short). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

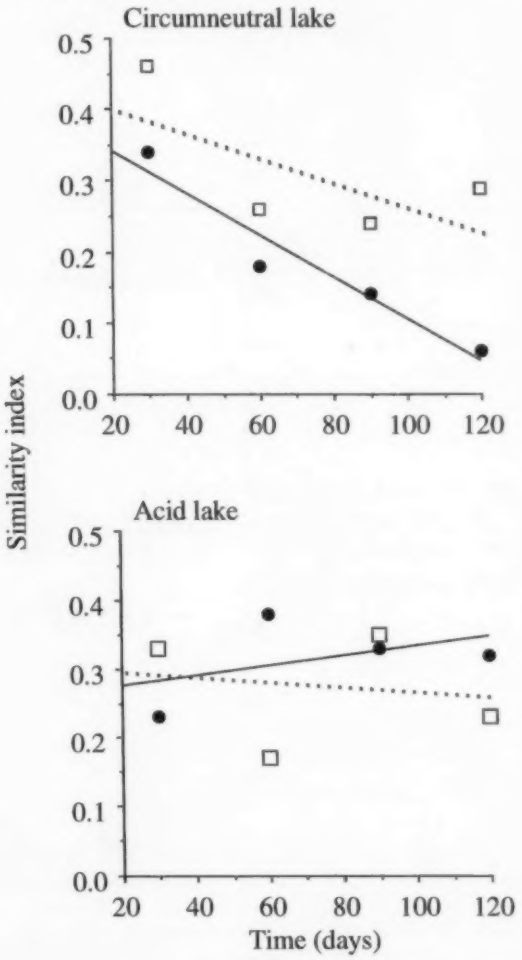
growth forms became increasingly more dominant over the course of the summer, replacing early successional and loosely attached forms that had been suppressed by grazing (Figs. 3 and 5). Differences in successional rates between the two lakes could not be attributed to differing habitat conditions, as the tiles were deployed at near vertical inclinations in sheltered bays of both lakes so as to insure comparable levels of minimal erosional stress associated with water flow. These long-term positive effects of grazers on periphyton succession may not be as evident during the earlier stages of periphyton development. Tuchman and Stevenson (1991) found that snail grazing did not consistently affect periphyton species succession over a 28-day period, while Lamberti et al. (1989) showed that snail grazing did delay succession over a 75-day period. Although herbivory-induced plant succession may not always be apparent at the individual species level, an examination of changes in the overall physiognomic composition of a community may reveal more distinctive successional patterns.

The exclusion of large (>5 mm) herbivores may also have reduced their physical disturbance of smaller (<5 mm) invertebrates on the tiles, potentially resulting in higher abundances of small herbivores (e.g., Cattaneo and Kalff 1986). Indeed, chironomid densities were twofold higher on tiles in the exclosures than on the open control tiles in the circumneutral lake (M.D. Graham, University of Regina, Regina, Sask., unpublished data). Despite such a greater abundance of potential small herbivores, periphyton biomass remained significantly higher on the tiles in the exclosures, except during September. Thus, larger grazers had a substantially greater overall impact than did the smaller grazers on periphyton in the circumneutral lake. Furthermore, this finding reflects how periphyton removal rates increase significantly with grazer body biomass (Cattaneo and Mousseau 1995).

Periphyton competition

Food-chain theory predicts that plant communities that are left unregulated by herbivores are increasingly regulated by competition for limiting resources. Therefore, competitive growth forms should dominate ungrazed periphyton communities. We found that tall growth forms tended to dominate ungrazed periphyton during midsummer in both the circumneutral lake

Fig. 5. Regression analyses showing successional rates for periphyton physiognomy on tiles from treatments with (+ grazers, solid line and circles) or without (– grazers; broken line and squares) exposure to large grazers in the circumneutral (solid line: $y = 0.34 - 0.003x$, $r^2 = 0.93$; broken line: $y = 0.4 - 0.002x$, $r^2 = 0.46$) and acid lakes (solid line: $y = 0.26 + 0.007x$, $r^2 = 0.21$; broken line: $y = 0.3 - 0.004x$, $r^2 = 0.03$).



and the acid lake where grazing was not apparent (Figs. 4 and 5). Tall stature can confer various competitive advantages to benthic algae, as competition (sensu Tilman 1982) is expected to be more intense lower down in the periphyton mat due to the vertical attenuation of light and nutrients (McCormick 1996). A tall stature can increase resource uptake rates by both increasing the surface area to volume ratio and extending above competing shorter growth forms. The increasing abundance of tall growth forms during succession on ungrazed tiles in the circumneutral lake and under relatively severe resource limitation in the acid lake supports how tall growth forms are favoured as competition is expected to intensify during periphyton development (McCormick 1996).

Loosely attached, tall growth forms were more abundant under severe resource limitation conditions, suggesting that

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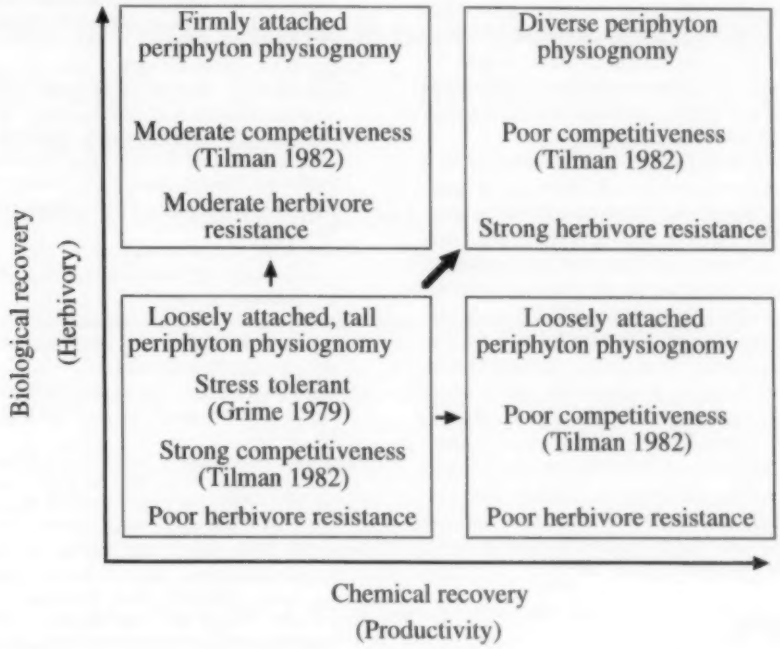
Fig. 6. Conceptual model showing trade-offs between functional responses of periphyton to increasing DIC and herbivory in recovering acidified lakes. Chemical recovery should stimulate poor competitors over stress-tolerant growth forms that are superior competitors for DIC. In contrast, biological recovery should favour herbivore resistance over competitiveness. Concomitant biological and chemical recovery should result in a highly diverse periphyton physiognomy.



they are stress-tolerant (sensu Grime 1979), strong competitors (sensu Tilman 1982) that flourish in highly acidified lakes owing to their ability to efficiently utilize low DIC concentrations. In a previous study (Vinebrooke 1996), abiotic conditions, such as DIC, dissolved organic carbon, and pH, best explained the composition of periphyton assemblages along an acidity gradient of lakes, further suggesting that stress tolerance and strong competitiveness are important traits of dominant physiognomic groups in highly acidified lakes. Conversely, loosely attached, tall periphyton also showed slow biomass accrual rates and the inability to exploit higher DIC levels in less acidic lakes (Fig. 4), traits that could be considered indicative of a weak competitor (sensu Grime 1979). Thus, an assessment of the competitiveness of loosely attached, tall periphyton in DIC-limited, acidified lakes is dependent on the definition of competition.

Differences in competitiveness amongst the various periphyton physiognomies could not be solely explained on the basis of morphological features. For example, resource availability had opposite net effects of firmly and loosely attached growth forms that shared a tall stature (Figs. 4 and 5). The different physiologies of zygnematacean and oedogoniacean taxa may have contributed to the differential responses of these two overstory growth forms to resource availability. Loosely attached zygnematacean taxa are highly efficient users of the limiting resource of DIC in acid lakes (Turner et al. 1995), while firmly attached oedogoniacean taxa may show higher nutrient-saturated uptake rates and growth potentials (Steinman 1996). Thus, morphological and physiological features should ideally both be considered in physiognomic assessments of competitiveness amongst periphyton (e.g., Rosemond 1996).

Trade-offs

Trade-offs between herbivore resistance and competitiveness among physiognomic groups of periphyton may involve physiological costs that are expressed as differences in growth rate (Rosemond 1996). Herbivore-resistant groups, such as firmly attached, short and tall growth forms, showed slower biomass accrual rates than did loosely attached, short taxa. In contrast, the latter group was composed of relatively fast-growing, early colonists that were subsequently suppressed by grazing in midsummer.

Physiological trade-offs between stress tolerance and competitiveness (sensu Grime 1979) could also be identified in this study. Acid-tolerant, loosely attached, tall growth forms showed relatively slow biomass accrual and were outcompeted by faster growing, loosely attached, short forms on ungrazed tiles in the more productive circumneutral lake (Fig. 4). However, loosely attached, tall forms were better able to tolerate stressors associated with growth in highly acidic lakes, such as metal toxicity and high ultraviolet irradiance (Vinebrooke and Graham 1997).

Regulation of periphyton physiognomy in recovering acid lakes

Chemical recovery in acidified lakes should affect periphyton competition through increasing resource availability and productivity (Fig. 6). Increasing DIC concentrations will suppress highly competitive (sensu Tilman 1982), loosely attached

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growth forms (i.e., zygnematacean taxa) that are less effective under higher DIC and nitrogen and phosphorus conditions (Fairchild and Sherman 1990; Vinebrooke 1996). Our results suggest that higher DIC levels and increased periphyton productivity should favour relatively less competitive (sensu Tilman 1982), loosely attached, understory growth forms (i.e., desmids, motile diatoms); however, concomitant increases in grazing pressure and habitat heterogeneity should offset their dominance and thereby enhance the diversity of periphyton in recovering acidified lakes.

Biological recovery in acidified lakes should largely be a function of chemical recovery and may be expected to cause increased grazing of periphyton due to recolonization by acid-sensitive herbivores (Fig. 6). We showed that benthic herbivory occurred in a circumneutral lake and was primarily attributable to green frog tadpoles, a species that is highly responsive to increases in lake alkalinity and pH (Glooschenko et al. 1992). Abundant crayfish populations in the acid lake did not significantly affect periphyton and may have indirectly released periphyton from grazing pressures by predation of smaller herbivores (e.g., Lodge et al. 1994). Higher grazing pressure in recovering acidified lakes should suppress competitive, overstory taxa in favour of herbivore-resistant, firmly attached growth forms (i.e., *Coleochaete scutata*, oedogoniacean taxa), precluding the characteristic proliferation of floating clouds of metaphytic zygnematacean algae in acidified lakes (Stokes 1986; Appelberg et al. 1993).

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DNA adducts and histopathological lesions in perch (*Perca fluviatilis*) and northern pike (*Esox lucius*) along a polycyclic aromatic hydrocarbon gradient on the Swedish coastline of the Baltic Sea

G. Ericson, E. Lindesjöö, and L. Balk

Abstract: DNA adducts, histopathological abnormalities, and organosomatic indices were used to study contaminant effects on fish along a polycyclic aromatic hydrocarbon (PAH) gradient, leading away from an aluminum smelter on the Swedish Baltic coast. The level of DNA adducts, analysed using the ^{32}P-postlabelling method, was at least 145 times higher in the liver of female perch (*Perca fluviatilis*) from the innermost site on the gradient, closest to the suspected point source of PAHs, than at a distant reference site. Of the DNA adducts analysed, a relatively small number accounted for a very high proportion of the total level of adducts (30–60% at the innermost site and close to 100% at the outermost site). These particular adducts could also be observed in extrahepatic tissues, such as trunk kidney and head kidney, along the entire gradient. Similar patterns of adducts were also observed in northern pike (*Esox lucius*). Focal hepatocellular degeneration in perch was about 15 times more extensive at the innermost site than at the next site in the gradient and absent in perch from the two outermost sites. Body size relative to age was also significantly reduced in perch from the three innermost sites compared with the outermost site.

Résumé : Nous avons utilisé les adduits d'ADN, les anomalies histopathologiques et des indices organosomatiques pour étudier les effets des polluants chez les poissons le long d'un gradient d'hydrocarbures aromatiques polycycliques (HAP) qui s'étend à partir d'une aluminerie située en Suède, sur la côte de la mer Baltique. La concentration d'adduits a été déterminée par la méthode du post-marquage au ^{32}P; la valeur mesurée dans le foie de perches (*Perca fluviatilis*) femelles provenant de la station la plus proche de la côte et de ce qu'on présume être la source ponctuelle de HAP, était au moins 145 fois supérieure à celle mesurée à la station de référence, située à distance. Un nombre relativement restreint de types d'adduits composait une très grande partie de l'ensemble des adduits mis en évidence (30–60% à la station la plus proche de la côte et presque 100% à la station la plus éloignée). Ces types se retrouvaient aussi ailleurs que dans le foie, notamment dans le rein antérieur et le rein troncal, et ce, sur toute la longueur du gradient. Une répartition semblable des adduits a aussi été observée chez le grand brochet (*Esox lucius*). Chez la perche, la dégénérescence hépatocellulaire focale était à peu près 15 fois plus répandue à la station la plus proche de la côte qu'à la station qui la suivait sur le gradient, et elle était inexistante aux deux stations les plus éloignées de la côte. Nous avons aussi constaté que le rapport taille–âge diminuait dans une mesure significative chez les sujets provenant des trois stations les plus proches de la côte, par comparaison aux sujets prélevés à la station la plus éloignée. [Traduit par la Rédaction]

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants derived mainly from incomplete combustion of organic matter. The presence of PAHs in the aquatic environment is of concern because of their toxic, mutagenic, and carcinogenic properties.

Many investigations of possible biological effects of environmental PAH contamination have relied on analysis of PAHs in sediments or water. However, these data do not give complete information about the extent and the rate at which these substances are bioavailable to fish and other organisms, or the toxicologic effects of such exposure.

Fish are able to metabolize PAHs into more hydrophilic products and thus detoxify many of them. However, detoxification is not the only process that occurs, as certain PAHs may be biotransformed into highly reactive intermediates that are able to bind covalently to cellular macromolecules and so exert their toxicity.

Analysis of DNA adducts has been suggested as a biomarker of exposure to PAHs, and several studies have demonstrated elevated levels of adducts in the livers of fish from areas with varying degrees of PAH contamination (Dunn et al. 1987; Varanasi et al. 1989; Maccubbin and Black 1990; Stein et al. 1992; Collier et al. 1993; van der Oost et al. 1994; El Adlouni et al. 1995). The level of cellular reaction products such as DNA adducts represents the integration of several pharmacokinetic processes: uptake, distribution, metabolism

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(including both activation and deactivation), excretion, DNA repair, and cell turnover. DNA adducts may thus serve as biomarkers of exposure and a dosimeter of the biologically effective dose in the target tissue investigated.

Histopathological abnormalities represent an integration of physiological and biochemical changes that themselves reflect the effects of exposure to anthropogenic pollutants. These abnormalities, being markers at a higher level of biological organization, have a greater ability to predict the eventual impact on the survival of the affected individual and therefore on the population (Hinton et al. 1992). Consequently, numerous field studies have attempted to show that fish being exposed to local sources of anthropogenic contaminants in coastal or inland waters exhibit histopathological and macroscopic pathological changes. As a result, these changes have been shown to be reliable biomarkers of effects of exposure to anthropogenic contaminants (Lindesjöö and Thulin 1994; Lindesjöö et al. 1994; Myers et al. 1994).

Chemical modification of DNA is generally accepted to be a critical step in chemical carcinogenesis and cytotoxicity, and there are several reports of liver lesions and other histopathological abnormalities in benthic fish species from contaminated areas (Vethaak et al. 1992; Myers et al. 1994). However, it is important to stress that other types of biological lesions may also result from exposure to genotoxic substances. Examples of ecologically significant effects include reduced embryonic viability and genetic disorders. It can be assumed that increased germline mutation rates as a consequence of exposure to mutagens will have deleterious or population-modifying effects in certain environments by decreasing fitness parameters in affected species (Würgler and Kramers 1992).

We used levels of DNA adducts in different tissues, and histopathological changes in the liver, as biomarkers of chemical exposure involving effects at different levels of biological organization. The study was performed on perch (*Perca fluviatilis*) and northern pike (*Esox lucius*) in an area of known PAH contamination on the Swedish Baltic coast. In a pilot study on perch from this area carried out in 1992, we found high levels of hepatic DNA adducts in the liver by comparison with perch from a reference area (Ericson et al. 1995). The objectives of the present investigation were to study (*i*) how far from the source of pollution DNA adducts could be detected in fish livers, (*ii*) whether or not patterns of DNA adducts similar to those found in the liver could also be detected in extrahepatic tissues, and (*iii*) whether or not the presence of DNA adducts was correlated with effects at a higher level of biological organization.

Materials and methods

Study area

The sea in the vicinity of the city of Sundsvall on the Swedish Baltic coast is heavily contaminated with PAHs. The largest point source of PAH emissions is an aluminum smelter, with an annual production of 100 000 t of aluminum (Landner 1990). Other sources of PAH emissions within the investigation area include traffic, local oil spills, and the heating systems of buildings in the municipality of Sundsvall which has about 90 000 inhabitants. Näf et al. (1994) estimated PAH flux to be about 560 kg/year in the 10-km^2 area of sea closest to the aluminum smelter. Total PAH concentrations (based on 15 compounds) varied between 5.3 and 41 μg/g dry mass (88–680 μg/g C) in settling particulate matter and between 14 and 211 μg/g dry mass (370–2700 μg/g C) in surface sediments (0–1 cm) on the sea bottom along a transect of about 10 km extending in a southeasterly direction from the aluminum smelter (Näf et al. 1994). The concentrations decreased with increasing distance from the emission point. Total PAH concentration in settling particulate matter at a reference site about 75 km north of the Sundsvall area was estimated in the same study to be 1.0 μg/g dry mass (14.3 μg/g C). Several other industries are also located in the area, including pulp and paper industries and a chemical industry. Together, they contribute to a very complex pollution load.

Water turnover in the area is rapid due to two large freshwater currents originating from river outflows and to upwelling of brackish water.

Biological material and tissue sampling

Perch and northern pike were caught with gill nets during October 1994, outside the spawning season, which for these species occurs during spring. After capture, fish were caged at each site (Fig. 1) for up to 1 week before tissue sampling was carried out. Site 1 was located about 1 km outside the smelter emission point, and the distance to sites 2, 3, and 4 was about 7, 12, and 28 km, respectively. The two outermost sampling locations are also exposed to the influence of the open sea. Fish were also caught in a reference area about 600 km away at Kvädöfjärden (58°00′N, 16°42′E), also located on the Swedish Baltic coast. Perch from this area have been used for anthropogenic effects monitoring in the Swedish National Environmental Programme for several years, with the results indicating exposure to comparatively low levels of anthropogenic substances (Balk et al. 1996). Nine to 13 perch and one to five northern pike were captured at the different sites in the Sundsvall area. At the reference site, 22 perch and 10 northern pike were captured. The majority of perch were females, and DNA adduct analysis and quantification of hepatocellular degeneration were carried out only on tissues from female fish. Nine randomly selected individuals of perch from the reference site were also analysed for DNA adducts. In northern pike, only analysis of DNA adducts was performed.

Total body length and total body mass of each sampled fish were recorded together with liver, spleen, and gonad mass. Fish condition index (FCI, somatic body mass expressed as a percentage of cubic length), gonadosomatic index (GSI, gonad mass expressed as a percentage of somatic body mass), liver somatic index (LSI, liver mass expressed as a percentage of somatic body mass), and spleen somatic index (SSI, spleen mass expressed as a percentage of somatic body mass) were calculated. Tissue sampling for DNA extraction was performed as follows. Three thin slices were cut out from the liver, one from the proximal end, one from the centre, and one from the distal end, respectively. From small perch, the whole liver apart from the slice used for histology was taken. Half the spleen, the left lobe of the head kidney, and an approximately 2-cm section of the central part of the trunk kidney from northern pike and the remaimder of the trunk kidney (after sampling for histology) in perch were also taken. Gill filaments were scraped off with a glass slide. About 4–5 cm of the small intestine was cut open, rinsed in 0.9% NaCl, and the epithelial cells scraped off with a glass slide. The whole brain was also collected. All tissues were immediately frozen in liquid nitrogen and thereafter stored at –80°C until DNA extraction was performed. Age of the perch was determined from the opercular bone (Le Cren 1947).

Histological preparation and analysis

A slice up to 3 mm in thickness was cut out from the central part of the liver of each specimen. Pieces of the same size were also collected from the spleen and the trunk and head kidneys. The central part of the second gill arch on the left-hand side was also collected. Tissues were processed, embedded, sectioned, and stained using routine histological procedures. Sections were numbered, with samples from

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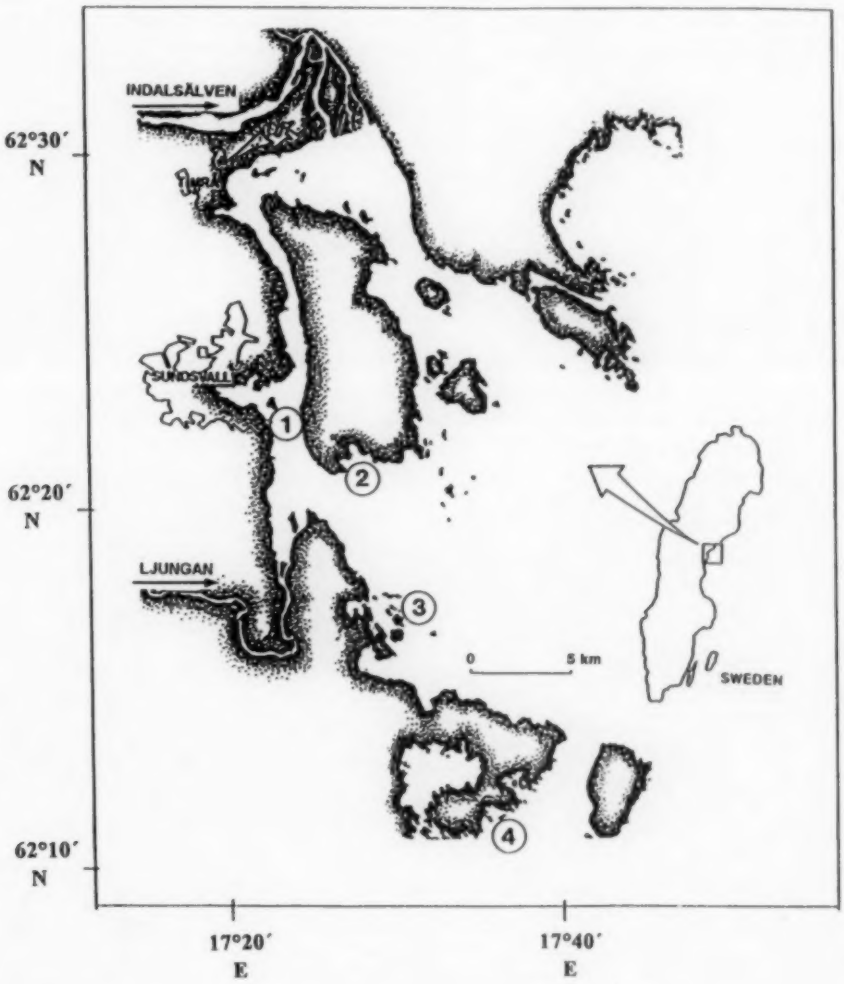
Fig. 1. Map showing sampling sites in the area outside the aluminum smelter together with the location of the study area on the Swedish Baltic coast.



different sites mixed together to ensure a blind, objective analysis. The area of each liver section was first measured by automatic detection with image analysis in a stereomicroscope (10×). Degenerative or necrotic areas were encircled manually on the computer screen with the computer mouse and measured at a magnification of 100×.

DNA purification

The DNA purification procedure was based on the methods of Dunn et al. (1987) and Reichert and French (1994). Between 10 and 100 mg of tissue (depending on the amount available) was homogenized with 1.5 mL of 50 mM Tris and 35 mM EDTA at pH 8.0 in a Dounce homogenizer. Homogenates were centrifuged in a Biofuge A centrifuge at 4°C for 10 min at 3200 × *g*. Pellets were resuspended in 700 µL of 50 mM Tris and 20 mM EDTA at pH 8.0, with 0.5% SDS and 1 mg proteinase K/mL, and incubated for 3 h at 37°C. Proteins were removed by sequential extraction with phenol, phenol – chloroform – isoamyl alcohol (25:24:1), and chloroform – isoamyl alcohol (24:1). Nucleic acids were precipitated by adding 0.1 volume of 5 M NaCl and 1 volume of –20°C ethanol and resuspended in 700 µL of 50 mM Tris and 20 mM EDTA at pH 8.0. Samples were incubated with 9.2 U of RNase A and 29 U of RNase T_1 for 30 min at 37°C. In the case of liver samples, 20 µg of α-amylase was included for hydrolysis of glycogen. A 400-µg amount of proteinase K in 40 µL of 50 mM Tris–HCl was added and incubation was continued for 30 min. Extraction with organic solvents was repeated, and DNA was precipitated with ethanol as described above. Purified DNA was dissolved in 10 mM Tris and 1 mM EDTA at pH 7.4 and quantified by its UV absorption at 260 nm, using a GeneQuant spectrophotometer (Pharmacia, Sweden).

^{32}P-Postlabelling analysis

In perch, analysis of DNA adducts was performed on purified DNA from liver, trunk kidney, and head kidney of individual fish. DNA adduct analysis was also performed on DNA extracted from gill filament, spleen, whole blood, brain, and intestinal mucosa. In these tissues, adducts were analysed using equimolar amounts of pooled DNA from five randomly selected individuals from each of sites 1 and 4. In northern pike captured at the different sites on the pollution gradient, DNA adduct analysis was carried out on pooled samples of equimolar amounts of DNA extracted from liver, gill, head kidney,

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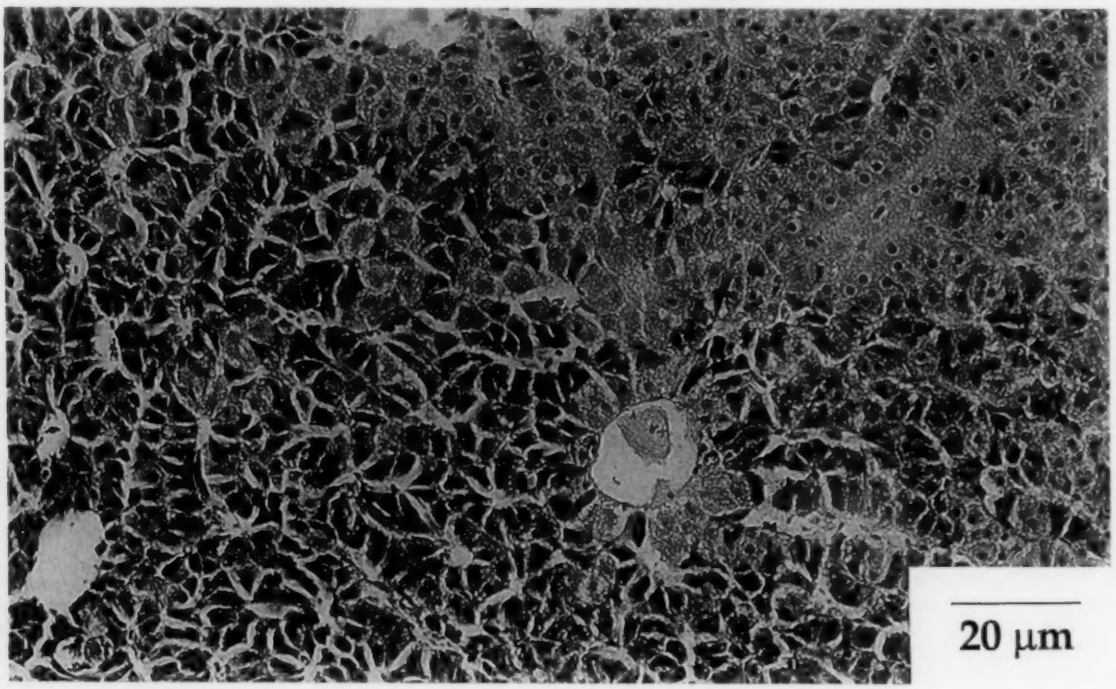
Table 1. Age, morphometric characteristics (±95% confidence intervals), somatic indices (fish condition index (FCI), gonadosomatic index (GSI), liver somatic index (LSI), and spleen somatic index (SSI)), and hepatocellular degeneration (HCD) of female perch from the reference site and sites along the pollution gradient in the Sundsvall area.

Variable	Site 1 ($n = 9$)	Site 2 ($n = 9$)	Site 3 ($n = 13$)	Site 4 ($n = 11$)	Reference site ($n = 22$)
Age (years+)	6.3±0.5	6.1±1.0	5.8±0.7	6.2±1.1	6.3±0.9
Total length (cm)	18.6±2.40 *b*	19.8±1.18 *b*	27.8±0.73 *a*	30.8±1.10 *c*	28.5±2.06 *ac*
Total mass (g)	74.0±29.4 *b*	85.0±14.0 *b*	240±18.3 *c*	330±37.9 *a*	324±83.1 *a*
FCI (%)	0.99±0.05 *b*	1.01±0.04 *b*	1.02±0.06 *b*	1.03±0.04 *b*	1.17±0.05 *a*
GSI (%)	7.65±1.48 *ab*	9.05±0.83 *b*	9.26±0.82 *b*	9.49±1.16 *b*	7.52±0.46 *a*
LSI (%)	1.98±0.43 *ab*	1.75±0.18 *ab*	1.88±0.17 *b*	1.89±0.15 *b*	1.50±0.08 *a*
SSI (%)	0.08±0.02 *a*	0.14±0.04 *b*	0.16±0.03 *b*	0.13±0.03 *b*	na
HCD (%)	1.074±1.279*	0.065±0.088	0.000	0.000	na

Note: Row means followed by different letters are significantly different from each other, having nonoverlapping 95% confidence intervals. *Significantly different from site 2; $p \leq 0.05$, Mann–Whitney *U*-test. na, not analysed.

Fig. 2. Liver of perch captured at the innermost site (site 1) along the pollution gradient in the Sundsvall area with focal hepatocellular degeneration in the upper right part of the photograph. Paraffin sections, 5 µm, hematoxylin and eosin.



and trunk kidney, females and males being treated together. For comparison, northern pike captured at the reference site were also analysed.

Adducts were analysed by ^{32}P-postlabelling (Gupta et al. 1982; Beach and Gupta 1992) after enrichment of aromatic and hydrophobic adducts by treatment with nuclease P1 (Reddy and Randerath 1986). Aliquots of 12.5 µg of DNA were hydrolysed to deoxynucleoside-3′-monophosphates with 65 mU of spleen phosphodiesterase and 300 mU of micrococcal endonuclease (P 6897 and N 3755, Sigma Chemical Co., St Louis, Mo.) for 4 h at 37°C in a total volume of 25 µL of 0.1 mM $CaCl_2$ – 10 mM succinate buffer at pH 6.0. Normal nucleotides were dephosphorylated by adding 1 µL of nuclease P1 (236 225, Boehringer Mannheim, Scandinavia AB, Sweden; 5.0 µg in 30 mM sodium acetate and 3.4 mM $ZnCl_2$ at pH 5.3) and incubating for another 45 min. Finally, pH was adjusted to 7.6 by adding 1 µL of 0.5 M Tris base. The mixture was evaporated to dryness and redissolved in 4 µL of distilled water.

The remaining nucleotides were labelled with ^{32}P in the 5′-position by adding 10 µL of a labelling mixture containing 8 U of T4 polynucleotide kinase (3′-phosphatase free, 838 292, Boehringer Mannheim, Scandinavia AB, Sweden), 3 MBq of [γ-^{32}P]ATP (≥185 TBq/mmol), 70 mM Tris base, 14 mM $MgCl_2$, 14 mM dithiothreitol, and 3.5 mM spermidine at pH 7.6. Incubation lasted for 30 min at 37°C.

DNA adducts were separated from remaining normal nucleotides and excess ATP by selective chromatography on PEI–cellulose

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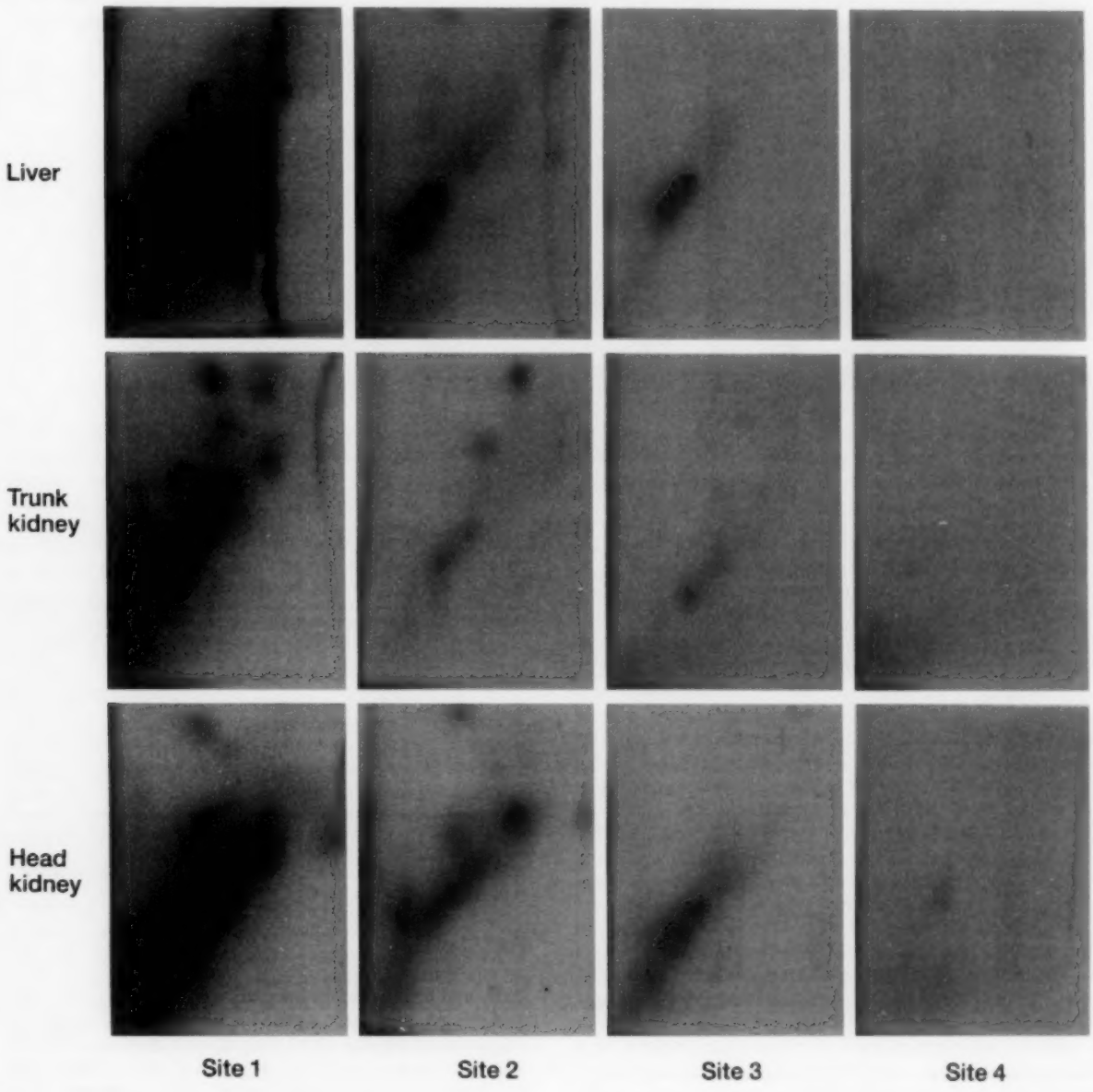
Fig. 3. Representative autoradiograms of chromatograms of ^{32}P-labelled digests of DNA from liver, trunk kidney, and head kidney of female perch captured at sites along the pollution gradient in the Sundsvall area. Autoradiography lasted 65 h at −80°C.



thin-layer chromatography (TLC) plates (Machery-Nagel, Germany). Adduct separation was made by contact transfer to new PEI plates and development in two dimensions (Lu et al. 1986). Chromatography solutions used were as follows: D1 = 1.0 M sodium phosphate, pH 6.8; D3 = 4.5 M lithium formate, 8.5 M urea, pH 3.5; D4 = 0.8 M lithium chloride, 0.5 M Tris, 8.5 M urea, pH 8.0; D5 = 1.7 M sodium phosphate, pH 6.0. Adducts were located by screen-enhanced autoradiography using Xomat AR-5 films (Eastman Kodak). The areas corresponding to ^{32}P-labelled adducts were excised from the TLC plates and radioactivity determined by liquid scintillation counting. Adduct frequencies were calculated using the net amount of radioactivity, the specific activity of the [γ-^{32}P]ATP used, and the relationship of 1 µg of DNA = 3240 pmol of nucleotides. Background correction was made by subtracting the amount of radioactivity on a similar area beside the adduct spots.

Salmon testis DNA (D 1626, Sigma Chemical Co., St. Louis, Mo.), treated in the same way as the samples, was used as a sample blank throughout the assays. DNA modified with benzo[*a*]pyrene in vitro was used as a positive control to monitor labelling efficiency between assays and as a chromatographic standard. Hepatic DNA from perch captured at site 1 was analysed twice, with an interval of 6 months between analyses. The mean divergence between the two

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Fig. 4. DNA adducts analysed by ^{32}P-postlabelling in liver, trunk kidney, and head kidney of female perch captured at sites along the pollution gradient in the Sundsvall area. Data derive from the analysis of nine individuals at each site and are expressed as means and 95% confidence intervals. **Significantly different from sites 2, 3, and 4; *significantly different from site 4; $p \leq 0.05$, analysis of variance followed by Tukey's test. At site 1, trunk kidneys of only two individuals were analysed (7.5 and 18.5 nmol adducts/mol nucleotides), and therefore, only the mean is given.

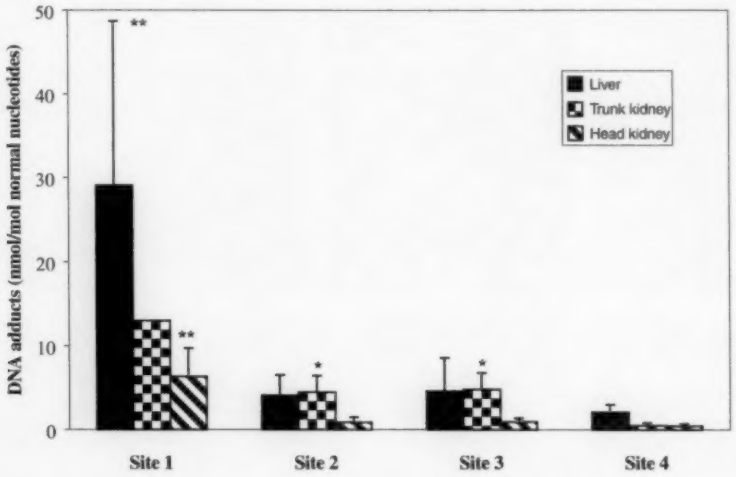
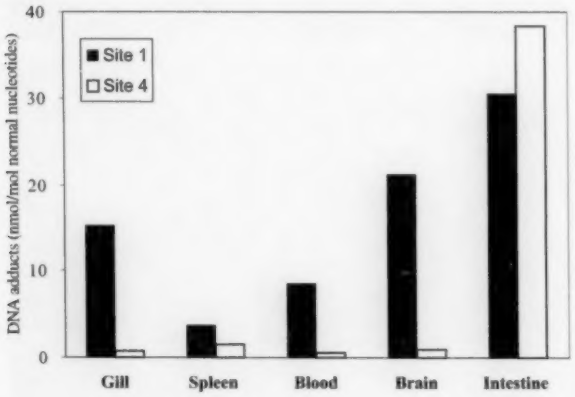


Fig. 5. DNA adducts analysed by ^{32}P-postlabelling in different tissues of female perch from the innermost site (site 1) and the outermost site (site 4) along the pollution gradient in the Sundsvall area. The analyses used pooled DNA from five individuals at each site.



sets of results was only 13.9%, indicating that interassay variation was small and that these adducts persist for at least 6 months in tissues stored at –80°C.

Statistical analysis

Mean values of variables derived from body and organ measurements were considered to be significantly different if the 95% confidence intervals did not overlap. Data on levels of DNA adducts were log-transformed and tested for homogeneity of variance using Cochran's test. Comparison between sites was made using one-way analysis of variance followed by Tukey's test. Histopathological abnormalities were compared among sites using the Mann–Whitney *U*-test. The level of significance in all these tests was set at $p \leq 0.05$.

Table 2. ^{32}P-Postlabelling analysis of DNA adducts in different tissues of northern pike from sites along the pollution gradient in the Sundsvall area and from the reference site.

	DNA adducts (nmol/mol normal nucleotides)			
Site	Liver	Gill	Head kidney	Trunk kidney
1	13.5 (1)	6.2 (1)	5.4 (1)	10.7 (1)
2	8.5 (1)	≤0.2 (1)	na	13.5 (1)
3	7.2±3.9 (5)	1.3 (5)	6.0 (5)	5.9 (5)
4	3.3±2.7 (3)	≤0.2 (3)	0.6 (3)	1.4 (3)
Reference	4.7±3.0 (10)	0.3±0.2 (6)	1.0±0.7 (6)	0.8±0.5 (6)

Note: Data for livers from sites 3 and 4 and all tissues from the reference site are means ± SD; all other data are from analysis of pooled DNA. Number of samples, or number of fish making up the pooled sample, given in parentheses. na, not analysed.

Results

Body and organ measurements

Total length, total mass, and SSI of female perch differed significantly among sites along the pollution gradient (Table 1). Although the average age of fish did not differ among sites, fish from sites 1–3 were significantly smaller than those from site 4, and fish from sites 1 and 2 were smaller than those from site 3. No significant differences in FCI and GSI were detected at sites 1–4. LSI values for female perch from the two outer sites on the gradient (sites 3 and 4) were significantly elevated in comparison with the reference site. SSI was significantly reduced in perch from the innermost site (site 1) in comparison with the three other sites in the Sundsvall area. No data on SSI were collected for fish from the reference site.

Histopathology

In perch from the two innermost sites from the Sundsvall area,

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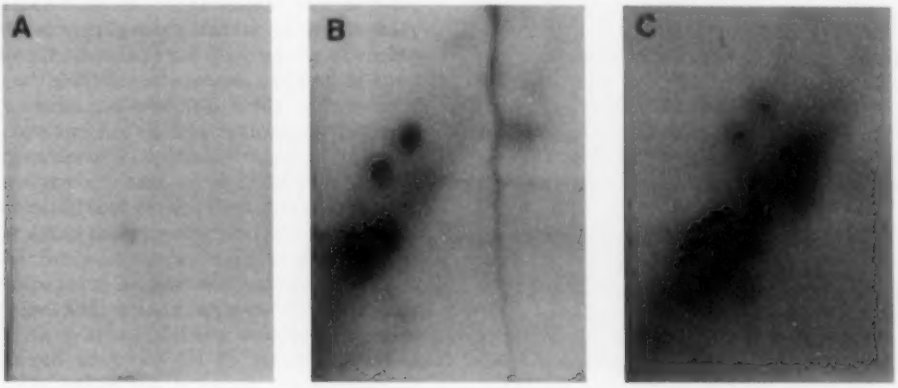
Fig. 6. Autoradiograms of chromatograms of digests of DNA from livers of female perch captured in 1994 at the (A) reference station (autoradiography for 64 h at –80°C) and (B) site 1 (48 h at –80°C) on the pollution gradient. (C) For comparison, an autoradiogram (52 h at –120°C) from the ^{32}P-postlabelling analysis of DNA adducts in the liver of a female perch captured at site 1 in 1992 is also shown. (With a shorter exposure time, the two main adduct spots become clearly visible within the diagonal radioactive zone.)



focal hepatocellular degeneration, i.e., clusters of cells without demarcations, was observed in the liver (Fig. 2). Degenerative areas were about 15 times more extensive at site 1 than at site 2 (Table 1). No hepatocellular degeneration was detected in perch from the two outermost sites. Some livers were also found with necrotic areas, but only to a minor extent. Inflammatory responses were absent. One specimen from site 1 exhibited very heavy vacuolization of the liver, with pycnotic hepatocytes. No other obvious histopathological abnormalities were found in any of the four organs examined.

DNA adducts

Adducts, in the form of several dark spots, could be observed on autoradiograms derived from liver, trunk kidney, and head kidney of perch from all capture sites along the pollution gradient (Fig. 3). The highest level of adducts was found in the liver at site 1. The level of adducts in all three tissues decreased along the gradient from site 1 to site 4 (Fig. 4). Adducts in the livers of perch from the reference area were at or below the method detection limit.

Adduct levels were higher in gill, spleen, blood, and brain of fish at site 1 than in the corresponding tissues of fish at site 4 (Fig. 5). The level of DNA adducts in the intestinal mucosa was, somewhat surprisingly, high at both sites and at similar levels to the liver.

In northern pike, levels of adducts in all tissues were lower at site 4 and the reference site than at site 1. Relative adduct levels at sites 2 and 3 varied, being similar to site 1 in kidney tissues, similar to site 4 and the reference site in the gill, and intermediate in the liver (Table 2).

Results of the DNA adduct analysis of perch livers from site 1 agreed with those of a study performed in the same area in 1992 (Fig. 6) when levels of between 6 and 22 nmol adducts/mol nucleotides were detected (Ericson et al. 1995).

Discussion

DNA adducts were detected in all perch tissues at the four sites along the pollution gradient. Total adduct levels were on average 10–20 times higher in all tissues (except intestine and spleen) at site 1 than at site 4. The patterns of adduct spots on the autoradiograms were similar in different individuals at the same capture site and to some extent also at different sites. Two particular spots could be seen on all autoradiograms from all sites along the gradient, making up 30–60% of the total level of adducts in the liver at site 1 and being the only spots detected at the outermost site. This indicates that despite many potentially genotoxic substances being present in the area, a few may account for the major part of the observed toxicity, and these few substances are distributed over a large area.

We made no attempt to determine the structure of the different adducts. However, the diagonal pattern of multiple overlapping spots observed in this study is characteristic of exposure to complex mixtures of PAHs. A similar pattern has been detected in several previous studies of fish from contaminated areas in different parts of the world (Dunn et al. 1987; Varanasi et al. 1989; Maccubbin and Black 1990; Stein et al. 1992; Collier et al. 1993; van der Oost et al. 1994; El Adlouni et al. 1995). Based on resistance to nuclease P1 treatment, and migration of adducts within the chromatographic system used, some general assumptions about the structure of the adducts can be made (Gupta et al. 1982; Reddy and Randerath 1986). Due to the high load of PAHs in settling particulate matter and sediments in the area (Näf et al. 1994), adduct resistance to nuclease P1 treatment, and adduct migration within the chromatographic system, we assume that the formation of at least some of the adducts can be attributed to exposure to PAHs.

The total adduct levels that we detected in perch are relatively low compared with field studies involving other species (Dunn et al. 1987; Varanasi et al. 1989; Maccubbin and Black 1990; Stein et al. 1992; Collier et al. 1993; van der Oost et al. 1994; El Adlouni et al. 1995). Besides interspecific differences in metabolizing enzyme systems, DNA repair, and other intracellular processes, differences in behaviour, such as feeding, may also be important. Perch, which inhabit the water column in the littoral zone, are not in close contact with sediment contaminants and thus experience less exposure than bottom-dwelling species. However, adduct frequencies in perch livers from the site closest to the aluminum smelter were at least 145 times higher than adduct levels at the reference

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site. This difference is much larger than the difference between contaminated and reference sites in most other studies and may indicate that we used a particularly clean reference area.

It is also important to emphasize that the ^{32}P-postlabelling assay is a semiquantitative method. The calculation of total adduct levels relies on the assumption of 100% labelling efficiency and adduct resistance to nuclease P1 treatment. Furthermore, depending on the elution system and the hydrophobicity of the adducts, some adducts may be retained at the origin on the TLC sheets or washed away and are thus not detected. Consequently, differences in the assay protocol used at different laboratories may lead to quite different levels of adducts.

Our findings of DNA adducts in several extrahepatic tissues of fish are the first to be reported in a field study. The liver exhibited the highest level of adducts, but high levels were also found in the gill, intestine, and brain of perch captured at the innermost site. The presence of adducts in the gills indicates that exposure takes place not only via the ingestion of food, but also via the passage of water over the gills. Balk et al. (1984) have shown that radiolabelled benzo[*a*]pyrene is associated with gill tissue to a much greater extent following water exposure than following dietary administration. In tissues rich in blood, some of the adducts detected could be derived from the blood. However, when adduct frequencies exceed those found in the blood, this cannot be the sole explanation. In all tissues examined, except for the intestine, adduct frequencies were higher in fish from the more contaminated site (site 1) than in fish from the less contaminated outermost site (site 4). In the intestine, high frequencies of adducts were found at both sites. We have no explanation for the high level of adducts in the intestine of fish from the outermost site. Work by James et al. (1991) demonstrated significant binding of radiolabelled benzo[*a*]pyrene in the intestine of southern flounder (*Paralichthys lethostigma*) following dietary administration of low doses. Hsu and Deng (1996) exposed zebrafish (*Brachydanio rerio*) to benzo[*a*]pyrene in the water and detected higher levels of adducts in intestine than in liver by ^{32}P-postlabelling. Thus, the intestine may be particularly sensitive to adduct formation.

It has been suggested that DNA adducts represent the cumulative result of long exposure to genotoxic compounds (Stein et al. 1992). Ray et al. (1995) detected DNA adducts in both liver and brain tissue of wild brook trout (*Salvelinus fontinalis*) from remote areas with no known input of anthropogenic contamination except possibly atmospheric fallout. Hepatic adduct levels were generally higher than levels in the brain. The authors suggested that brain tissue is less exposed to adduct-forming compounds due to its low xenobiotic metabolizing activity and that observed adduct frequencies in the brain may be the result of accumulation of nonrepaired adducts formed during long-term exposure to low levels of genotoxic substances. Accumulation of adducts over time may also explain the high frequencies that we detected in the brain of perch from the innermost site. Further field studies involving fish of different age groups and laboratory experiments using different exposure routes are needed to evaluate whether and how DNA adducts in different tissues could be used as biomarkers to distinguish between relatively recent and long-term exposure.

Although only a few northern pike from the contaminated area were examined in this study, the general pattern of DNA adducts was similar to the pattern found in perch. PAHs are not considered to biomagnify in the food chain, and thus, northern pike, being piscivorous, are not expected to be substantially more exposed to PAHs via the diet than are perch. Total adduct levels in northern pike from the Sundsvall area were in same range as those in perch. On the other hand, adduct levels in northern pike from the reference site were higher than in perch. The adduct patterns were, however, quite different from the pattern obtained from fish captured in the Sundsvall area. This indicates that adducts in northern pike from the reference site probably derive from exposure to other substances that possibly accumulate in the food chain. Juvenile northern pike from a small humus-rich pond in the south of Sweden, without any known input of anthropogenic substances, exhibited no detectable DNA adducts in either the liver or extrahepatic tissues such as kidney, gill, intestine, and brain (authors' unpublished data).

The prevalence of hepatocellular degeneration was increased in perch from the two innermost sites. In addition, morphometric quantification by computer-assisted image analysis showed that perch from the most contaminated site (site 1) had more severe lesions (expressed as percentage of liver tissue affected by hepatocellular degeneration) than perch from site 2. The type of hepatocellular degeneration described in this study, namely focal clusters of hepatocytes without any apparent demarcations between cells and in general without inflammatory responses, has recently been found in livers of rainbow trout (*Oncorhynchus mykiss*) exposed to different concentrations of bleached kraft mill effluent (authors' unpublished data). Similar histological changes can often be seen in the marginal tissues of histological sections and are interpreted as fixation artifacts. However, it should be stressed that to avoid this potential pitfall, the outer areas of all histological sections were excluded from the morphometric analysis carried out in the present study. This may in fact have led to an underestimation of hepatocellular degeneration.

Although ages were similar, the body size (length and mass) of female perch was less at the inner sites than at either site 4 or the reference site. There is no reason to suspect that the decreased body size was due to lower food availability or consumption. In fact, earlier studies in the Sundsvall area and at several other locations along the Baltic coast showed no large intersite variation in stomach contents of perch (Hansson 1987). Hargis et al. (1984) reported no weight gain in spot (*Leiostomus xanthurus*) experimentally exposed to PAH-contaminated sediment. However, near-acute toxic concentrations were used in that study. In the present study, relative spleen weight was also low in perch from the innermost site. As far as we are aware, SSI has not previously been measured in studies of the effects of anthropogenic stress on fish. However, Halbrook et al. (1993) observed a decrease in relative spleen weight in muskrats (*Ondatra zibethicus*) from a PAH-contaminated area. In the present study, LSI was higher at the two outer sites along the pollution gradient than at the reference site. LSI at site 1 (1.98 ± 0.43) was not significantly different from LSI at the reference site (1.5 ± 0.08); this was associated with large variation among individuals at site 1. Earlier studies have shown that the LSI of female perch at this age is normally between 1.4 and 1.6% (Balk et al. 1996). The degenerative lesions observed in the liver may have

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contributed to the large variation in LSI at the innermost site in this study. Gonad development in perch is rather rapid at the time of the year when sampling was performed, which probably explains the lower value of GSI at the reference site sampled 2 weeks earlier than the other sites. No significant differences in GSI were detected at sites 1–4 along the pollution gradient. However, the small gonad size in relation to the age of female perch at the innermost stations may indirectly have had an impact on reproduction.

In summary, this study has demonstrated the use of biomarkers at different levels of biological organization. We found indications of environmental stress at all levels: molecular, cellular, tissue, and whole organism. The effects were greater nearer the aluminum smelter. We believe that PAHs in effluents from the smelter are major contributors to adduct formation in fish from the area. PAHs are probably also, at least in part, responsible for the occurrence of histopathological lesions and for the observed effects on body and organ size.

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Lungworm (Nematoda: Metastrongyloidea) infections in Canadian phocids

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Abstract: *Otostrongylus circumlitus* (Railliet, 1899) was found in 5% (16/308) of grey seals (*Halichoerus grypus*), 6% (1/17; intensity = 38) of harbour seals (*Phoca vitulina*), and none of 100 harp seals (*Phoca groenlandica*) from eastern Canada and none of 31 ringed seals (*Phoca hispida*) from Holman, Northwest Territories. Eighty-two percent of these infections were observed in young-of-the-year seals. *Filaroides* (*Parafilaroides*) *gymnurus* (Railliet, 1899), detected in nodules in the superficial parenchyma of the lungs, infected 24% (5/16) of grey seals, 27% (4/15) of harbour seals, 57% (29/51) of harp seals, 81% (25/31) of ringed seals, and one stranded bearded seal (*Erignathus barbatus*) (new host report for harp, ringed, and bearded seals; new locality report for Holman). *Filaroides* (*Parafilaroides*) *hispidus* Kennedy, 1986 was found in systematically sliced lungs of grey seals (2/3; new host report) and ringed seals (2/7) but not in harp seals ($n = 11$) or harbour seals ($n = 5$). Intensity ranged from 37 to 3570 for *F.* (*P.*) *gymnurus* and from 295 to 1196 for *F.* (*P.*) *hispidus*. No detrimental effect on body condition of seals could be associated with infection by lungworms.

Résumé : *Otostrongylus circumlitus* (Railliet, 1899) a été trouvé dans 5% (16/308) des phoques gris (*Halichoerus grypus*), 6% (1/17; intensité = 38) des phoques communs (*Phoca vitulina*) et aucun des 100 phoques du Groenland (*Phoca groenlandica*) de l'est du Canada et aucun des 31 phoques annelés (*Phoca hispida*) de Holman, Territoires du Nord-Ouest. Quatre-vingt-deux pourcent de ces infections ont été observées chez des jeunes phoques de l'année. *Filaroides* (*Parafilaroides*) *gymnurus* (Railliet, 1899), détecté dans des nodules dans le parenchyme en surface des poumons, infectait 24% (5/16) des phoques gris, 27% (4/15) des phoques communs, 57% (29/51) des phoques du Groenland, 81% (25/31) des phoques annelés et un phoque barbu (*Erignathus barbatus*) échoué (nouvelle mention d'hôte pour le phoque du Groenland, annelé et barbu; nouvelle mention géographique pour Holman). *Filaroides* (*Parafilaroides*) *hispidus* Kennedy, 1986 a été trouvé à l'examen systématique de poumons de phoques gris (2/3; nouvelle mention d'hôte) et de phoques annelés (2/7), mais non dans ceux de phoques du Groenland ($n = 11$) ou de phoques communs ($n = 5$). L'intensité variait de 37 à 3 570 pour *F.* (*P.*) *gymnurus* et de 295 à 1 196 pour *F.* (*P.*) *hispidus*. Aucun effet préjudiciable sur la condition corporelle des phoques ne put être associé avec l'infection par ces nématodes pulmonaires.

Introduction

Two metastrongyloid genera, *Otostrongylus* de Bruyn, 1933 and *Filaroides* (*Parafilaroides*) (Dougherty, 1946) Anderson, 1978, are restricted to pinnipeds (Anderson 1978). Species of *Filaroides* (*Parafilaroides*) are found in most species of seals in the northern circumpolar region and have also been reported in pinnipeds of the Southern Hemisphere (review in Gosselin 1995). The monotypic *Otostrongylus circumlitus* (Railliet, 1899) de Bruyn, 1933 has been reported in pinnipeds from the North Atlantic Ocean (northern Europe, New England, and the eastern Canadian Arctic) and from both sides of the North Pacific Ocean (as far south as California on the western coast of North America) (review in Gosselin 1995).

Otostrongylus circumlitus, a large nematode in the bronchi and bronchioles, has its anterior extremity embedded in lung tissue with the tail (posterior extremity) extending anteriorly in lumina of airways, occasionally reaching the trachea (Onderka 1989). The anterior attachment of these large nematodes affects only a small proportion of respiratory tissue, but their presence obstructs airways, induces the production of mucus, and may lead to secondary bacterial infections (Stroud and Dailey 1978; van der Kamp 1987; Onderka 1989). Such lungworm infections occur mostly in young pinnipeds (Onderka 1989; Claussen et al. 1991). The life cycle of *O. circumlitus* is unknown, but Bergeron et al. (1997) have demonstrated that *O. circumlitus* may use fish as intermediate hosts.

Species of *Filaroides* (*Parafilaroides*) are small nematodes completely embedded within lung tissue, and their detection requires a meticulous examination of the parenchyma with the aid of a dissecting microscope (Onderka 1989; Borgsteede et al. 1991) or the use of specialized procedures such as the Baermann technique (as used by Claussen et al. 1991). Pathology associated with species of *Filaroides* (*Parafilaroides*) in pinnipeds is believed to be due to a hypersensitive immune response from the host (Geraci and St. Aubin 1987). These pathological responses have been observed predominantly in young stranded pinnipeds, which had secondary bacterial infections (Sweeney 1974; van der Kamp 1987). Occasional focal reaction to *Filaroides* (*Parafilaroides*) spp. has been observed in ringed seals (*Phoca hispida*) (Phocidea) and Australian sea lions (*Neophoca cinerea*) (Otaridea), and severe pathology has been reported in the northern elephant seal (*Mirounga angustirostris*) (Stroud and Dailey 1978; Nicholson and Fanning 1981; Onderka 1989; Gulland et al. 1997).

Filaroides (*Parafilaroides*) *decorus* is the only metastrongyloid

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infecting seals for which a life cycle is known. Dailey (1970) showed that *F.* (*P.*) *decorus* in California sea lions (*Zalophus californianus*) can use a small tidepool fish, *Girella nigricans*, as an intermediate host to complete transmission.

The importance of lungworms in seals in Canadian waters has not been examined. This study, therefore, had three objectives: (*i*) to identify the species of lungworms infecting seals in the estuary and Gulf of St. Lawrence and the Canadian Arctic, (*ii*) to determine prevalence and intensity of each species of lungworm, and (*iii*) to determine whether the presence of lungworms has an effect on seal body condition.

Materials and methods

Five different species of seals ($n = 457$) were collected from the Northwest Territories and eastern Canada during July 1987 to May 1994 (Fig. 1). Seals were collected under permit, accidently caught in fishing gear, or were found stranded. Age of each animal was determined by counting growth layer groups (GLGs) in thin sections of a canine tooth taken from the lower jaw (McLaren 1958; Mansfield 1991).

The trachea, lungs, and heart were removed and processed fresh, or immediately frozen and then thawed prior to examination. The four chambers of the heart were examined macroscopically. Each lung was weighed and the external surface was examined for visible nodular lesions. Observed lesions were counted, removed by cutting around the perimeter, and rinsed in saline. Each piece of this tissue was then pressed between glass plates and examined for worms with a dissecting microscope (Leitz M3, 10–40×) using transmitted light. This was the first method used to detect small embedded nematodes.

Major airways and chambers of the heart were rinsed with 100 mL of tap water which was stored at 4°C until examined for larvae. Major airways were opened and examined macroscopically for worms. Mucus remaining in opened airways was scraped and added to the bronchial rinse solution. These lavages were filtered through 500-µm sieves (Canadian Standard Sieve series No. 35, W.S. Tyler Canada, St. Catharines, Ont.) and the resulting solution was centrifuged at 2500 rpm (1020 × *g*) for 5 min in 250-mL bottles (GSA rotor in centrifuge model Z510, Berthold Hermle Gmbh & Co., Gosheim). The supernatant was discarded. The volume of the sediment was determined to the nearest 0.1 mL and suspended in 0.5–5.0 mL of tap water. A 1-mL sample of this suspension was spread on a glass plate and examined for the presence of larvae using a dissecting microscope (40×). One drop of this suspension was also examined with a compound microscope (Leitz Diaplan, 250×). Differences in prevalence of larvae between species of seals were tested using chi-square.

A second systematic method was used on 26 seals to detect small embedded lungworms within the parenchyma. Lungs in saline were frozen (–40°C) in deep trays and sufficient saline was added to completely contain the lungs in an ice block. Each lung encased in ice was cut transversally every 5 mm from the cranial apex to the caudal apex using a band saw (model 5212, Hobart Canada Inc., Don Mills, Ont.). Beginning at the cranial apex, and with the starting slice being randomly chosen from the first five slices, every fifth consecutive slice was systematically collected. Due to the width of the blade, this sample corresponded to 17% of the total lung as verified by mass. Sampled slices were thawed in saline, pressed between glass plates, and examined with a dissecting microscope (25–40×) using transmitted light. Detected worms were then removed carefully from tissue and cleaned in saline.

Lungworms belonging to *Filaroides* (*Parafilaroides*) embedded in lung parenchyma tended to coil upon themselves forming knots. These knots, which contained one or more nematodes, were sometimes delimited by a zone of white tissue which is hereafter called a nodular lesion associated with *Filaroides* (*Parafilaroides*) spp. For each lung systematically examined, the total number of embedded worms was obtained by the product of the number of knots observed, the mean number of worms per knot, and the factor 5.88 (100/17). To estimate the total number of worms in lungs of a seal, estimates from each lung were added, or multiplied by 2, when only one lung was examined. When two species of *Filaroides* (*Parafilaroides*) were found, estimates of each were obtained by the total number of worms found, multiplied by the proportion of each species of *Filaroides* (*Parafilaroides*) found in the extracted and identified worms. The position of each knot in slices was defined as central (on inner half of a straight line from the center of the slice to the surface and passing through the knot), peripheral (outer half of the same line), or at the surface when the knot was actually in contact with the pleura of the lung.

Embedded nematodes were extracted by tearing the surrounding parenchymal tissue in saline, using fine forceps and needles. When needed, the cuticle of nematodes was cleaned with soft paintbrushes. All nematodes (adults and larvae) used for morphological studies were fixed in hot 10% glycerin–alcohol (one part glycerin to nine parts 70% alcohol) and cleared by evaporation of the alcohol. Lactophenol was also used to clear some larger specimens. Worms mounted in glycerin on glass slides were studied and measured with a compound microscope (Leitz Diaplan) equipped with a drawing tube interfaced with a digitizer and computer.

Infection in seals was expressed as prevalence and mean intensity, based on detection of nodular lesions associated with worms on surface examination, and based on detection of embedded worms using systematic examination. Differences in prevalence of lungworms or nodular lesions between species or samples of seals were examined using a chi-square test (using frequencies) followed by the simultaneous test procedure (*G*-test, Scherrer 1984) or using Fisher's exact test (Scherrer 1984). Differences in mean intensity of lungworms or nodular lesions of *Filaroides* (*Parafilaroides*) spp. between species or samples of seals were examined using a Mann–Whitney *U*-test when two species or samples were compared and using Kruskal–Wallis followed by the procedure proposed by Noether (1976) when more than two species or samples were compared and a significant difference was found (see Scherrer 1984).

Differences between species of seals with respect to the percentage of gravid female nematodes were tested using Fisher's exact test. Differences between species of seals with respect to percentage of knots in which gravid females were present were tested using chi-square (using frequencies) followed by the simultaneous test procedure (*G*-test, Scherrer 1984).

The effect of lungworm infections on condition was examined by comparing body measurements and indices of body condition for infected seals versus uninfected seals. For each seal from eastern Canada, the following body measurements were taken: total body mass, standard length, axillary girth, maximum girth, sternal blubber thickness, and dorsal blubber thickness as described by the American Society of Mammalogists (1967). The sculp, which includes the skin and attached blubber from a point midway between the eyes and ears back to the base of the hind flippers, was removed and weighed. Subsequently, sculp mass (kilograms)/standard length (centimetres) × 100 and sculp mass (kilograms)/total body mass (kilograms) × 100 were used as indices of condition (Stewart and Lavigne 1984; McLaren and Smith 1985; Beck et al. 1993*b*). The measurements and condition indices of infected versus noninfected seals were compared for animals of the same age collected at the same sites and time of year using a Mann–Whitney *U*-test.

Results

Three species of lungworms, *O. circumlitus*, *Filaroides* (*Parafilaroides*) *gymnurus* (Railliet, 1899) Anderson, 1978, and *Filaroides* (*Parafilaroides*) *hispidus* Kennedy, 1986, were found in the lungs of seals ($n = 457$ (only 119 were examined

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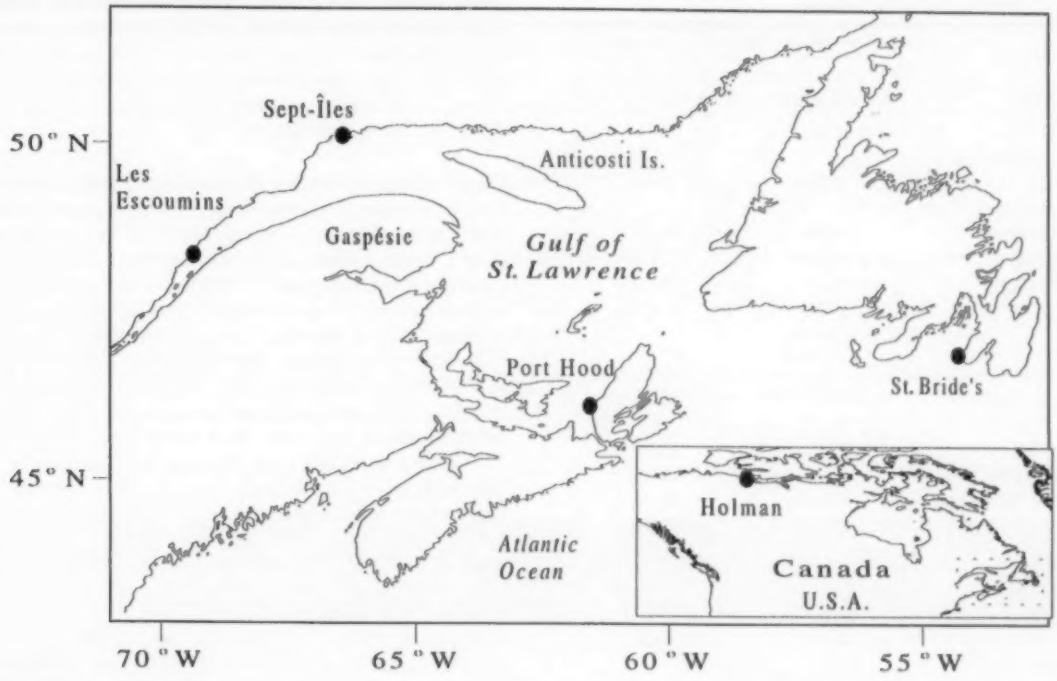
Fig. 1. Sites of collection of seals from eastern and Arctic Canada.



for *Filaroides* (*Parafilaroides*) spp.)) collected in eastern Atlantic and western Arctic Canadian waters (Tables 1 and 2; Figs. 2 and 3).

Otostrongylus circumlitus

Otostrongylus circumlitus, the largest of the three species of lungworms, was found only in harbour seals (*Phoca vitulina*) and grey seals (*Halichoerus grypus*) from the estuary and Gulf of St. Lawrence, showing no difference in prevalence between the two species of seals (Fisher's exact test, $p = 0.38$; Table 1). The infected harbour seal had 38 adult *O. circumlitus*: 12 males (with sclerotized spicules and developed bursa) and 26 females (with patent vulva and first-stage larvae in utero). Intensity was not determined in grey seals from Anticosti Island. There was no difference in prevalence between sexes of grey seals (Fisher's exact test, $p = 0.08$).

Otostrongylus circumlitus was found mostly in young seals, and 82% (14/17) of infected seals were young-of-the-year (age-class 0) (Fig. 4). The infected harbour seal, found stranded on May 24, 1994, was just completing its first year. In grey seals, prevalence of *O. circumlitus* was six times higher for the 0+ age-class (31%, $n = 45$) than for all age-classes combined (5%, $n = 308$). The youngest seal infected with *O. circumlitus* was 6.5 months old, was a grey seal collected on July 26, 1987, and was likely born in early January.

No first-stage larvae of *O. circumlitus* were observed in bronchial lavages, but no bronchial lavages were performed for seals infected with adult *O. circumlitus*.

Filaroides (*Parafilaroides*) spp.

Filaroides (*Parafilaroides*) spp. were more prevalent than *O. circumlitus*, occurring in all five species of seals and from all sites where seals were examined for these small lungworms, except Gaspésie where only three seals were examined (Table 1). These small lungworms embedded in lung parenchyma were not identified to species during surface examination of the lungs. All samples for each species of seal were combined to compare differences in prevalence between species of host, since no difference in prevalence of *Filaroides* (*Parafilaroides*) spp. between samples from different sites and seasons was observed (Fisher's exact test, $p = 0.16$–0.71; Table 1). Prevalence of nodular lesions associated with *Filaroides* (*Parafilaroides*) spp. was higher in species frequenting Arctic waters than in species frequenting only southern waters ($\chi^2 = 21.2$, df = 3, $p < 0.05$). *Filaroides* (*Parafilaroides*) spp. was more prevalent in ringed seals from the Arctic than in grey seals ($G = 17.3$) and harbour seals ($G = 12.7$) from eastern Canada, but no difference in prevalence was observed between Arctic ringed seals and migratory harp seals (*Phoca groenlandica*) ($G = 5.5$) which seasonally frequent Arctic and eastern Canadian waters. No differences in prevalence were observed between species of seals frequenting the estuary or Gulf of St. Lawrence when all samples of seals species were combined ($\chi^2 = 7.0$, $\alpha' = 0.0085$, df = 1, $\alpha = 0.05$; harp and grey seals $G = 6.8$, harp and harbour seals $G = 4.4$, grey and harbour seals $G = 0.04$). The only difference in prevalence between species in southern waters was found at Les Escoumins and agreed with the above, since nodular lesions were more prevalent in harp seals frequenting the Arctic in summer than in harbour seals which are year-round residents of the estuary and Gulf of St. Lawrence (Fisher's exact test, $p < 0.05$; Table 1).

Systematic examination of 26 seals previously examined for nodular lesions on the surface of the lungs revealed two

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Table 1. Lungworm (Metastrongyloidea) infections in seals collected in eastern and Arctic Canada.

	% prevalence (number examined)	
Host species	*O. circumlitus*	*Filaroides* (*Parafilaroides*) spp.[a]
Grey seal		
Les Escoumins, Qué. (May)	0 (5)	40 (5)
Anticosti Island, Qué. (July–Sept.)	6 (287)	na[b]
Port-Hood, N.S. (Jan.)	0 (15)	20 (15)
Sept-Îles, Qué.[c]	0 (1)	0 (1)
Total	5 (308)	24 (21)[d]
Harp seal		
Les Escoumins, Qué. (Dec.–Mar.)	0 (83)	71 (34)
St Bride's, Nfld. (Apr.–June)	0 (12)	33 (12)
Sept-Îles, Qué. (May)	0 (4)	25 (4)
Gaspésie, Qué. (Apr.)	0 (1)	0 (1)
Total	0 (100)	57 (51)[e]
Harbour seal		
Les Escoumins, Qué. (June)	0 (13)	31 (13)
Sept-Îles, Qué.[c]	0 (1)	0 (1)
Gaspésie, Qué. (May–Aug.)	33 (3)	0 (1)
Total	6 (17)	27 (15)
Ringed seal		
Holman, N.W.T. (Mar.–Apr.)	0 (31)	81 (31)[e]
Bearded seal (*Erignathus barbatus*)		
Gaspésie, Qué. (Aug.)	0 (1)	100 (1)

[a]Based on presence of nodular lesions associated with *Filaroides* (*Parafilaroides*) spp. on the surface of lungs. All new geographic records for *F.* (*P.*) *gymnurus*.

[b]Seals were not examined for *Filaroides* (*Parafilaroides*) spp.

[c]Seals were collected in spring or summer.

[d]New host record for *F.* (*P.*) *hispidus*.

[e]New host record for *F.* (*P.*) *gymnurus*.

species of *Filaroides* (*Parafilaroides*). *Filaroides* (*Parafilaroides*) *gymnurus* was found in grey (2/3), harp (4/11), harbour (3/5), and ringed seals (7/7), while *F.* (*P.*) *hispidus* was identified only in grey (2/3; new host report) and ringed seals (2/7). *Filaroides* (*Parafilaroides*) *gymnurus* was more prevalent than *F.* (*P.*) *hispidus* in seven ringed seals examined systematically (7/7 versus 2/7, respectively; Fisher's exact test, $p = 0.01$). No difference was observed in the intensity of *Filaroides* (*Parafilaroides*) spp. infections among species of seals using either the number of nodular lesions or the number of worms ($H = 6.3$, df = 3, $p = 0.10$ and $H = 0.9$, df = 3, $p = 0.83$, respectively; Table 2).

Infections with *Filaroides* (*Parafilaroides*) spp. were more evenly distributed over all age-classes than infections with *O. circumlitus* (Fig. 4). However, nodular lesions associated with *Filaroides* (*Parafilaroides*) spp. were most common in younger animals for harp seals divided by age groups (age 0, ages 1–3, age 4, and older; $\chi^2 = 6.4$, df = 2, $p < 0.05$). The mean intensity of *Filaroides* (*Parafilaroides*) spp. nodular lesions did not differ among age groups. Between sexes, only harp seals showed a higher number of nodular lesions associated with *Filaroides* (*Parafilaroides*) spp. in males (mean ± SD = 15.7 ± 16.9, $n = 17$) than in females (mean ± SD = 5.8 ± 7.1, $n = 9$; $Z = -2.1$, $p < 0.05$).

Observations on species of *Filaroides* (*Parafilaroides*)

During systematic examination of lungs, the reproductive status of worms was assessed. All *F.* (*P.*) *gymnurus* and all *F.* (*P.*) *hispidus* extracted from tissue were adults (males had sclerotized spicules and females had a patent vulva with or without larvae). The percentage of knots containing *Filaroides* (*Parafilaroides*) spp. that bore gravid females was 74% (316/426) in ringed seals, 50% (62/124) in harbour seals, 46% (34/74) in grey seals, and 30% (74/244) in harp seals. Gravid females of *Filaroides* (*Parafilaroides*) spp. were more frequent in ringed seals than in all other species of seals ($G = 22–125$, $p < 0.05$) and more frequent in harbour seals than in harp seals ($G = 14$, $p < 0.05$; $\chi^2 = 127$, df = 3, $p < 0.05$). In knots where worms were identified as *F.* (*P.*) *gymnurus*, the percentage of knots with gravid females was 72% (18/25) in ringed seals, 55% (43/78) in harp seals, 20% (1/5) in harbour seals, and 14% (1/7) in grey seals, with a statistical difference occurring only between ringed and grey seals (Fisher's exact test, $p < 0.05$). For *F.* (*P.*) *hispidus*, this percentage was 84% (16/19) in ringed seals and 71% (10/14) in grey seals (Fisher's exact test, $p = 0.23$).

First-stage larvae of *Filaroides* (*Parafilaroides*) spp. were found in lavages of the bronchi and bronchioles of 96 seals (Table 3). The prevalence of larvae did not differ significantly between species of infected seals (Fisher's exact test, $p = 0.12–0.21$ or $\chi^2 = 0.67$, df = 1, $p = 0.42$; Table 3). No larvae were found in lavages of 30 seals having infections of adult *Filaroides* (*Parafilaroides*) spp. (seven of which had gravid females). In contrast, larvae were present in lavages of harp seals in which no adult infections were found (Table 3).

Information on the position in the lung and association with white nodular lesions of both species of *Filaroides* (*Parafilaroides*) during systematic examination allows a comparison with examination of the surface of lungs for nodular lesions. *Filaroides* (*Parafilaroides*) *gymnurus* was found in parenchyma, while *F.* (*P.*) *hispidus* was found in parenchyma and in conjunctive tissue surrounding bronchi and bronchioles. The proportion of knots (worms with no tissue reaction and worms within white nodular lesions) that were in contact with lung pleura was low in both species of worms (5% for *F.* (*P.*) *gymnurus* and 3% for *F.* (*P.*) *hispidus*). However, the proportion of knots that exhibited a white nodular lesion was 49% (67/136) for *F.* (*P.*) *gymnurus* but only 8% (3/38) for *F.* (*P.*) *hispidus* (Fisher's exact test, $p < 0.01$). Of the 21 surface nodular lesions from grey seals and ringed seals from which worms were extracted and identified, only *F.* (*P.*) *gymnurus* was found.

Effect of lungworm infections on seals

Otostrongylus circumlitus was found with the head (anterior extremity) embedded in lung parenchyma and the tail (posterior extremity) projecting into the lumen of bronchioles and bronchi as far anteriorly as the bifurcation of the trachea. Firm,

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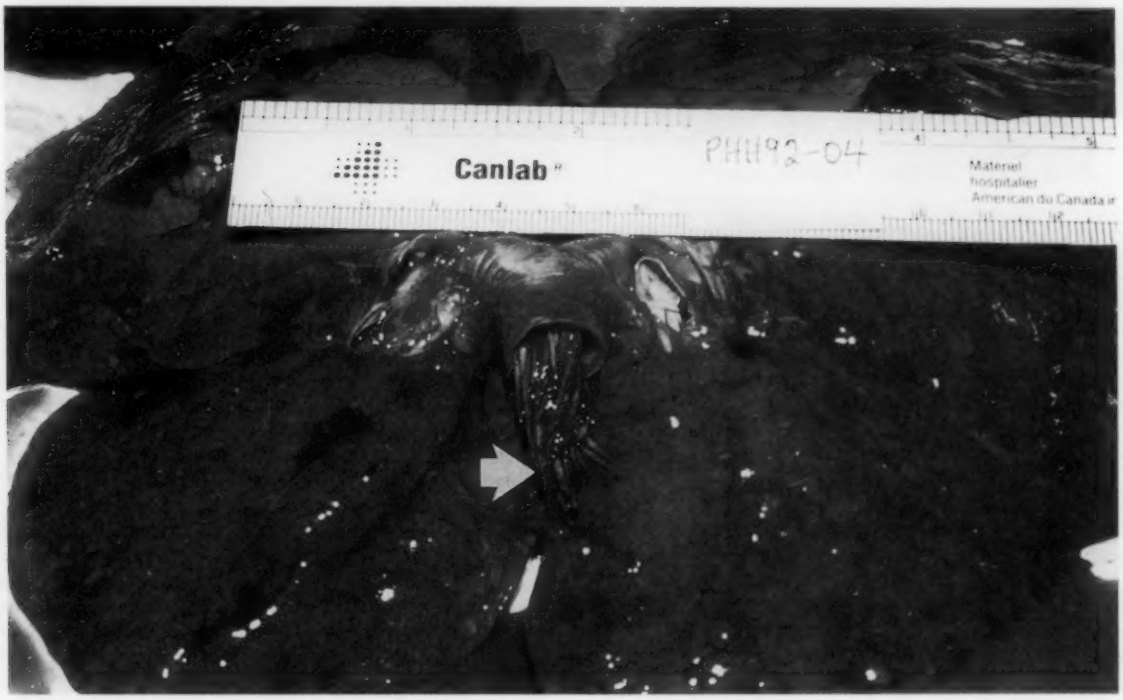
Table 2. Intensities of *Filaroides* (*Parafilaroides*) spp. in seals from Canadian waters using positive surface nodular lesions and systematic analysis of lungs.

		Surface nodular lesions[a]		Systematic worm count	
Seal species	Worm species	n^b	Mean intensity ± SD (range)	n^b	Mean intensity ± SD (range)
Grey seal	*F.* (*P.*) *gymnurus*	5	3±2 (2–6)	3	139±148 (37–308)
	F. (*P.*) *hispidus*			2	314±26 (295–332)
Harp seal	*F.* (*P.*) *gymnurus*	26	12±15 (1–51)	4	646±314 (184–847)
	F. (*P.*) *hispidus*			0	—
Harbour seal	*F.* (*P.*) *gymnurus*	4	21±31 (3–68)	3	680±561 (35–1056)
	F. (*P.*) *hispidus*			0	—
Ringed seal	*F.* (*P.*) *gymnurus*	25	27±50 (2–228)	7	896±1285 (71–3570)
	F. (*P.*) *hispidus*			2	1040±219 (886–1196)

[a]Numbers based on surface nodular lesions do not differentiate between *F.* (*P.*) *gymnurus* and *F.* (*P.*) *hispidus*.
[b]Number of seals infected with worms used to calculate mean intensity.

Fig. 2. Seventy-seven *O. circumlitus* in the bronchi and bronchioles of a young-of-the-year ringed seal collected in Salluit, Québec, in September 1992. Results presented in Measures and Gosselin (1994).



yellow lesions up to 1 cm^3 were observed in lung parenchyma around attached anterior extremities of *O. circumlitus* with abundant thick, yellow mucus in associated airways.

Effects of *O. circumlitus* infections in young-of-the-year grey seals from Anticosti Island were determined using indices of condition (Table 4). Only sculp mass/body mass × 100 showed a lower value for infected seals, but no significant difference was observed.

The significant differences associated with infection with *Filaroides* (*Parafilaroides*) spp. showed that infected seals were in better body condition than uninfected seals. Harp seals in their first year (age-class 0) collected at St Bride's, Newfoundland, in April–June, with nodular lesions associated with *Filaroides* (*Parafilaroides*) spp. ($n = 3$), were significantly longer (117 ± 2 versus 106 ± 5 cm) and heavier (39 ± 1 versus 29 ± 3 kg) and had heavier sculp masses (16 ± 1 versus 12 ± 1 kg) and higher values of total mass/standard length × 100 (33 ± 1 versus 27 ± 2 kg/cm), sculp mass/standard length × 100 (13.7 ± 0.5 versus 11.7 ± 0.6 kg/cm), and maximum girth^2/standard length (59 ± 3 versus 53 ± 3 cm) than uninfected ones

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Fig. 3. Nodular lesions associated with *Filaroides* (*Parafilaroides*) spp. on the surface of the lungs of a harp seal collected in Les Escoumins, Québec, March 1992.

($n = 7$; Mann–Whitney U-test, df = 8, $p < 0.05$). Furthermore, adult harp seals (age-class 4 and older) collected at Les Escoumins, Québec, from December through March, infected with nodular lesions associated with *Filaroides* (*Parafilaroides*) spp., had a significantly (Mann–Whitney U-test, $p < 0.05$) thicker dorsal layer of blubber (5.6 ± 1.0 cm, $n = 14$) than did uninfected animals (4.7 ± 1.0 cm, $n = 8$).

Discussion

Two genera of metastrongyloids comprising three species were found in five species of seals examined from eastern Canadian and western Arctic Canadian waters.

Infections with *O. circumlitus* occurred almost exclusively (82%) in young-of-the-year seals. The higher susceptibility of younger seals was also observed by Stroud and Dailey (1978), Smith et al. (1979), Onderka (1989), Borgsteede et al. (1991), Claussen et al. (1991), and Measures and Gosselin (1994). The scarcity of infections in age-classes 1+ and older may be due to (*i*) changing diets (older animals may no longer be exposed to infective stages in prey items, e.g., a higher proportion of crustaceans in the diet of younger individuals has been reported for harp and ringed seals (Sergeant 1973; Lowry et al. 1980)), (*ii*) development of protective immunity (once exposed to infection, animals recover and cannot be reinfected), (*iii*) mortality (infected young seals do not recover and are eliminated from the population (Geraci 1977)), and (*iv*) age resistance (a refractory response to the parasite develops naturally as the animal matures, even with no previous infection, as suggested by Urquhart (1990)).

The life cycle of *O. circumlitus* is unknown, but the absence of infections in very young animals suggests that this parasite is acquired through the food chain when infective larvae are ingested with infected intermediate hosts, as seen in other metastrongyloids (Anderson 1978). The youngest infected seal in the present study was a 6.5-month old grey seal. The youngest infected ringed seal examined by Onderka (1989) was 3.5 months old. Measures and Gosselin (1994) observed infected ringed seals as young as 4 months. Bronchopneumonia associated with lungworms was found in 35% (27/77) of stranded seals (mostly harbour seals) older than 3 months, but was absent in 50 seals younger than 3 months in The Netherlands (van der Kamp 1987).

Evaluation of *O. circumlitus* infections in a population must be based on samples of seals within the susceptible age of 3.5 months to 1 year old. In the present study, the number of seals within this age range was 45 for grey seals, five for harp seals, and three for harbour seals. No age was available for ringed seals, but based on length (see Smith 1987), no ringed seals were within this susceptible age range for infection with *O. circumlitus*. This could explain that prevalence (10% or less) in this study was low compared with the reported 78% in harbour seals from The Netherlands and 30% in harbour seals from New England (van den Broek 1963; Geraci 1977). However, this latter value is similar to the 31% observed in young-of-the-year grey seals. The Netherlands and New England samples were likely biased toward higher values, since they were composed of stranded and sick animals with high proportions of young individuals. However, our study, based mostly on hunted seals, may include a bias toward

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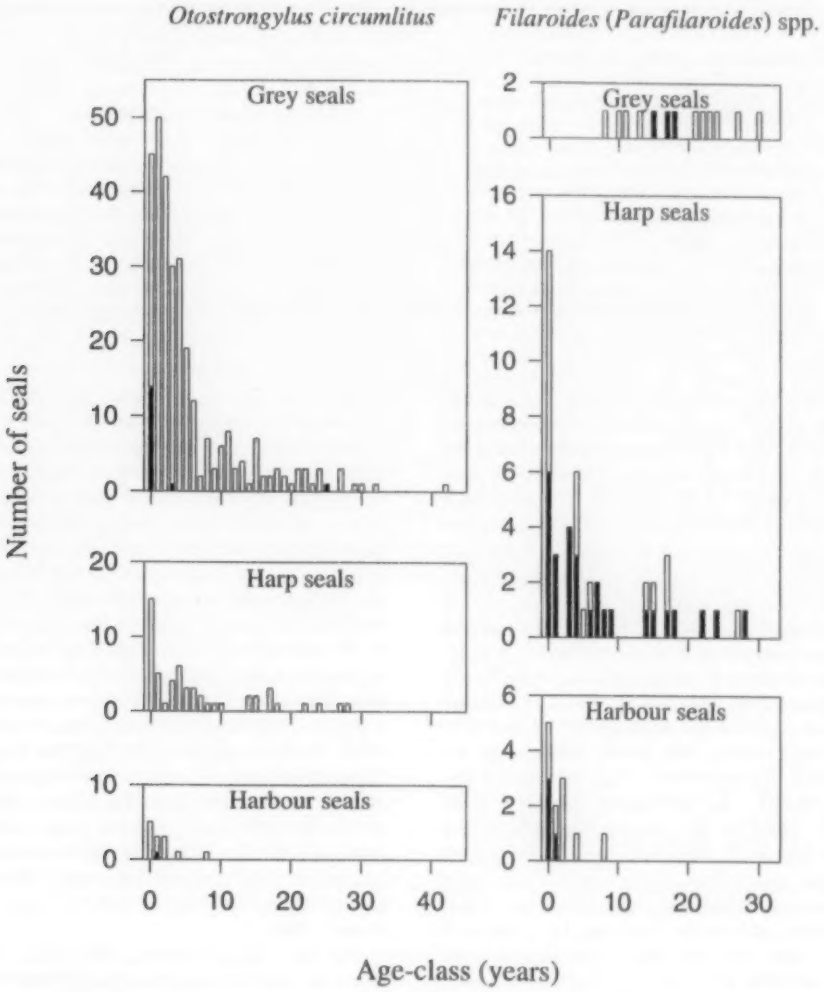
Fig. 4. Age distribution of seals collected in eastern Canada and examined for *O. circumlitus* and *Filaroides* (*Parafilaroides*) spp. Infections are shown in black.



young seals, since young seals are naive and may be less wary of hunters, and a bias toward infected seals, since they may need longer recovery time at the surface than uninfected seals.

Filaroides (*Parafilaroides*) *gymnurus* was found in five species of seals and in all geographic areas from which seals were collected, and lungs were examined for embedded nematodes (Table 2). Infections of *F.* (*P.*) *gymnurus* in ringed and harp seals represent new host records. The presence of *F.* (*P.*) *gymnurus* in seals from western Arctic Canada and eastern Atlantic Canadian waters also represents new geographic records. *Filaroides* (*Parafilaroides*) *hispidus* was found only in ringed seals from Holman and grey seals from Les Escoumins. The observation of *F.* (*P.*) *hispidus* in grey seals from eastern Atlantic Canada represents new host and geographic records for this species which was first described from ringed seals from western Arctic Canada (Kennedy 1986). The report of *Filaroides* (*Parafilaroides*) *kraschenin-nikovi* in ringed seals from Salluit (Measures and Gosselin 1994) was changed to *F.* (*P.*) *gymnurus* with the synonymy proposed by Gosselin and Measures (1997).

Table 3. Presence of *Filaroides* (*Parafilaroides*) spp. larvae using lavages of airways of seals from Canadian waters.

	% prevalence (number examined)		
Seal species	All seals lavaged	Adults	No adults
Grey seal	0 (10)	0 (2)	0 (8)
Harp seal	26 (43)	38 (24)	11 (19)
Harbour seal	14 (14)	50 (4)	0 (10)
Ringed seal	34 (29)	43 (23)	0 (6)

Note: All seals lavaged are also divided according to the presence of adult worms in surface nodular lesions.

Infections of *Filaroides* (*Parafilaroides*) spp. were distributed in all age-classes within different species of seals. Prevalence of *Filaroides* (*Parafilaroides*) spp. in the present study

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Table 4. Effects of *O. circumlitus* infections on young-of-the-year grey seals collected on Anticosti Island in summer 1987 and 1992.

	Infected		Uninfected		
Measurement or index of condition	*n*	Mean ± SD	*n*	Mean ± SD	p^a
Body mass (kg)	14	49±6	31	45±8	0.07
Standard length (cm)	14	132±18	31	125±9	0.29
Axillary girth (cm)	14	88±13	31	83±6	0.22
Maximum girth (cm)	13	89±13	31	86±6	0.52
Sternal blubber thickness (cm)	5	2.4±0.4	5	2.4±0.5	1.00
Dorsal blubber thickness (cm)	9	2.3±0.7	26	2.0±0.5	0.17
Sculp mass (kg)	14	15±2	31	15±3	0.68
Sculp mass (kg)/standard length (cm) × 100	14	12±2	31	12±2	0.98
Sculp mass (kg)/body mass (kg) × 100	14	31±5	31	33±4	0.11

[a]Mann–Whitney *U*-test.

was higher in juvenile harp seals than in young-of-the-year harp seals. A similar trend was observed in *F.* (*P.*) *gymnurus* from harbour seals from The Netherlands which showed the highest prevalence in 1- to 2-year-old seals (Borgsteede et al. 1991) and also observed in *F.* (*P.*) *hispidus* in ringed seals from the western Arctic Canada which showed highest prevalence in the middle age-classes (Onderka 1989). The four possible explanations given for the absence of *O. circumlitus* in old age-classes may also apply here.

Although transmission and biology of *F.* (*P.*) *gymnurus* and *F.* (*P.*) *hispidus* are unknown, these worms could be better adapted to Arctic ecosystems or Arctic phocids. This would explain the higher prevalence observed in ringed seals compared with grey seals and harbour seals which, for the most part, do not enter Arctic waters. This would also account for the intermediate prevalence observed in harp seals which frequent the Arctic in summer and the estuary and Gulf of St. Lawrence in winter. Although physiological or behavioural differences between species of hosts may have an impact on prevalence, Yurakhno and Popov (1976), in a study using helminths to differentiate ringed seal populations, found that *F.* (*P.*) *krascheninnikovi* was more prevalent in a northern sample (Bering strait: 12%, $n = 187$) than in a southern sample (Anadyr Gulf: 5%, $n = 59$). Moreover, if one examines the prevalence of *Filaroides* (*Parafilaroides*) (either *F.* (*P.*) *hispidus* or *F.* (*P.*) *gymnurus* (=*F.* (*P.*) *krascheninnikovi*) (see Gosselin and Measures 1997)) in ringed seals found in the present study and that reported in the literature, it appears that prevalence generally decreases in samples from Arctic Canada (Holman: 81%, $n = 31$, present study; Beaufort Sea (Holman): 53%, $n = 79$) to the Chukchi Sea (27%, $n = 60$) and into the Bering Sea (11%, $n = 228$) (Yurakhno and Skrjabin 1971; Onderka 1989). However, these observed differences may be due to differences in sample sizes, sampling and detection techniques, season of collection, and transmission dynamics in each geographic location. Often the age of infected seals is not reported. Nevertheless, species of *Filaroides* (*Parafilaroides*) appear to be widespread in the Arctic in ringed seal populations.

Life histories of lungworms and food habits of their hosts could explain differences in prevalence observed between species of seals. Life histories of lungworms in pinnipeds, however, are not well known, but *F.* (*P.*) *decorus* in California sea lions, which is the only metastrongyloid of pinnipeds for which a life cycle is known, uses a tidepool fish (*G. nigricans*) as an intermediate vertebrate host (Dailey 1970). Higher prevalences of *Filaroides* (*Parafilaroides*) spp. (likely *F.* (*P.*) *gymnurus*) in ringed and harp seals, which feed predominantly pelagically (McLaren 1958; Lowry et al. 1980), compared with grey seals, which feed predominantly benthically (see Benoit and Bowen 1990), and harbour seals, which feed benthically and pelagically (Boulva and McLaren 1979), suggest that pelagic transmission of *Filaroides* (*Parafilaroides*) spp. in Canadian waters is more efficient than benthic transmission. If fish are used as intermediate hosts, Arctic cod (*Boreogadus saida*) could play a significant role in transmission in Arctic Canadian waters where it forms an important component of the diet of ringed and harp seals (McLaren 1958; Lowry et al. 1980; Finley et al. 1990). When harp seals come to the St. Lawrence estuary in winter, they feed almost exclusively on capelin (*Mallotus villosus*) (Beck et al. 1993*a*). An intermediate host specificity (i.e., capelin, Arctic cod) could explain the lower prevalences in grey and harbour seals which have less specialized diets composed of a great diversity of fish species and invertebrates (Boulva and McLaren 1979; Benoit and Bowen 1990).

The percentage of female *Filaroides* (*Parafilaroides*) spp. attaining maturity and producing larvae could be an index of host suitability. Gravid female *F.* (*P.*) *gymnurus* were found in all seals regardless of season of collection, indicating that this species matures in five species of seals. Female *F.* (*P.*) *hispidus* matured in grey and ringed seals. Ringed seals showed a higher percentage of knots with gravid female *Filaroides* (*Parafilaroides*) spp. bearing larvae than all other species of seals. Higher prevalence and higher percentage of gravid female *Filaroides* (*Parafilaroides*) spp. in ringed seals compared with other species of seals suggest that ringed seals are more suitable hosts.

Surprisingly, the only significant differences observed in measurements and indices of condition suggested that young-of-the-year harp seals infected with *Filaroides* (*Parafilaroides*) spp. were larger or were in better condition than uninfected seals. This contrasts with observations by van den Broek (1963), who associated the presence of *F.* (*P.*) *gymnurus* with serious disease and mortality. Nevertheless, these data support the idea that *Filaroides* (*Parafilaroides*) spp. are transmitted through the food chain, as has been demonstrated for *F.* (*P.*) *decorus* (Dailey 1970). Infected harp

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seals found to be in better body condition may represent newly infected young individuals that resumed feeding earlier or more intensively after the postweaning fast.

No detrimental effect on body measurement or indices of condition could be associated with *O. circumlitus* infections. Onderka (1989) suggested that infected young-of-the-year seals do not survive *O. circumlitus* infections, as no worms or lesions suggesting previous infections were observed in older age-classes. Secondary bacterial infections may occur with lungworm infections (Stroud and Dailey 1978). If such infections provoke serious morbidity and lead to mortality of infected young-of-the-year seals, one would expect to see a deterioration in condition of infected seals. Smith (*in* McLaren and Smith 1985) mentioned that condition indices based on blubber thickness and girth failed to confirm statistically the visual observation that juvenile ringed seals infected with *O. circumlitus* appear smaller. The reduction of respiratory parenchyma associated with the attachment of *O. circumlitus* may be limited, but numerous worms and copious mucus may occlude the bronchial lumina (Onderka 1989). This would reduce gas exchange efficiency and would increase recovery time at the surface after a dive. Smith et al. (1979) noted that seals infected with *O. circumlitus* appear to make shorter dives, which would affect their feeding success and increase their vulnerability to predation. Infections with *O. circumlitus* could have a direct (pathological lesions) or an indirect effect (increased risk of predation) on survival of young seals, which could subsequently reduce recruitment in seal populations.

The difficulty in demonstrating the effect of a pathogenic parasite on host condition is, in part, a function of intensity and duration of infection (acute or chronic). There are little data on prepatent period of pinniped lungworms (see Dailey 1970) and no data on patent period, longevity of infections, or pathogenesis. Consequently, large samples of seals from the field or experimental data covering the entire infection period are required to demonstrate convincing significant effects on seal condition by lungworm infections.

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Methyl mercury dynamics in littoral sediments of a temperate seepage lake

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Abstract: The sites and rates of methyl mercury (MeHg) production and transport in littoral zone sediments were investigated at Pallette Lake in northern Wisconsin. In littoral areas where groundwater inflow occurs, sulfate supply from groundwater creates profiles of electron acceptors (sulfate) and donors (methane, sulfide) that are reversed from those found in sediments whose sulfate supply is delivered from overlying water. The highest MeHg concentrations in porewaters and the maximal advective MeHg flux rates (4.5–61.7 $ng \cdot m^{-2} \cdot day^{-1}$) were observed in the spring, while highest bulk phase concentrations occur later in the summer. These estimated MeHg fluxes are greater than the mean areal production rates estimated previously for the water column and are similar to the atmospheric flux. Gross MeHg production was measured using the addition of ^{203}Hg as a tracer to sediments. The depth at which maximal ^{203}Hg methylation occurred coincided with the observed maximums in solid-phase and porewater MeHg concentrations. Because input, advection, and accumulation of MeHg in these sediments were measured directly, an independent estimate of MeHg production could be made and compared with ^{203}Hg-derived rates. This comparison suggests that that the ^{203}Hg tracer method provides reasonable estimates of gross methylation rates and that a substantial fraction of solid-phase Hg is available for methylation.

Résumé : On a étudié les sites et les taux de production et de transport du méthyl mercure (MeHg) dans les sédiments de la zone littorale au lac Pallette, dans le nord du Wisconsin. Dans les zones littorales où il y a afflux d'eau souterraine, l'apport de sulfate provenant de l'eau souterraine crée des profils d'accepteurs (sulfate) et de donneurs (méthane, sulfure) d'électrons qui sont inversés par rapport à ceux observés dans les sédiments qui reçoivent l'apport de sulfate des eaux de surface. Les plus fortes concentrations de MeHg dans les eaux interstitielles et les taux maximaux des flux d'advection du MeHg (4,5–61,7 $ng \cdot m^{-2} \cdot jour^{-1}$) ont été observés au printemps, tandis que les plus fortes concentrations dans la phase solide des sédiments ont été observées plus tard dans l'été. Ces flux estimés du MeHg sont supérieurs aux taux moyens de production par unité de surface estimés antérieurement pour la colonne d'eau, et sont similaires au flux atmosphérique. On a mesuré la production brute de MeHg en ajoutant aux sédiments du ^{203}Hg à titre de traceur. La profondeur à laquelle a eu lieu la méthylation maximale du ^{203}Hg a coïncidé avec les maximums observés des concentrations de MeHg dans la phase solide et dans l'eau interstitielle. Étant donné que l'afflux, l'advection et l'accumulation du MeHg dans ces sédiments ont été mesurés directement, une estimation indépendante de la production de MeHg a pu être réalisée et comparée aux taux dérivés à partir du ^{203}Hg. Cette comparaison fait ressortir que la méthode utilisant le ^{203}Hg comme traceur fournit des estimations raisonnables des taux bruts de méthylation, et qu'une fraction substantielle du Hg de la phase solide est susceptible d'être méthylée. [Traduit par la Rédaction]

Introduction

As clean techniques have become standard practice in aquatic mercury (Hg) studies, investigators have focused efforts on important biogeochemical processes and cycling pathways of Hg. Of particular interest has been an examination of external monomethyl mercury (MeHg) sources to aquatic ecosystems such as precipitation (St. Louis et al. 1995), runoff (Swain et al. 1992; St. Louis et al. 1994; Hurley et al. 1995), and groundwater (Krabbenhoft et al. 1995), as well as internal processes that result in MeHg production in the water column (Watras et al. 1995) and at the sediment–water interface (Gilmour et al. 1991; Krabbenhoft et al. 1995; Branfiren et al. 1996). Recent research emphasis placed on the production, transport, and fate of MeHg results from (*i*) the observation that virtually all Hg in fish tissue is MeHg (Bloom 1992) and (*ii*) the recent development of techniques for measuring MeHg concentrations (Horvat et al. 1993) and in situ Hg methylation rates (Gilmour and Riedel 1995).

Recent studies have shown that system-scale parameters such as lake size and depth, littoral zone area, and epilimnetic temperature, as well as profile measurements of methylation rates determined on incubated cores, all point to the sediment–water interface in littoral zones as an important site for MeHg production (Bodaly et al. 1993; Ramlal et al. 1993). These observations corroborate with previous findings that sandy

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littoral zone sediments are a key site for sulfate reduction (Rudd et al. 1986) and that sulfate-reducing bacteria are important Hg methylating agents in aquatic ecosystems (Gilmour et al. 1991). Littoral sediments are also the dominant MeHg production site in recently flooded reservoirs (Hecky et al. 1991; Rudd 1995; Kelly et al. 1997). Many of the previous studies, however, have not taken into account the role that groundwater plays in the aquatic Hg cycle, particularly in littoral zones of lakes where groundwater discharge is generally focused. Not recognizing the direction and mode of aqueous transport in sediments can lead to erroneous conclusions.

Groundwater has two potentially important functions in the MeHg production process in aquatic ecosystems: as a transport vector and as a solute source for many biogeochemical processes. Advective transport of solutes species that might stimulate methylation (e.g., sulfate) usually dominates over diffusion in sandy sediments (Berner 1980) and dictates the ultimate direction of flow, either upward discharge to the lake or downward recharge to the contiguous aquifer. Unless the direction of groundwater flow is determined in sediment–water exchange studies, the source or sink function of the sediment–water interface cannot be fully ascertained. Groundwater can also provide solutes that are essential for many biogeochemical processes operating near the interface, such as dissolved organic carbon (DOC), inorganic Hg, and sulfate for the methylation process (Krabbenhoft et al. 1995). However, there have been few detailed examinations of the littoral zone sediment–water interface as a MeHg generation site in places where there is appreciable groundwater flow. The challenge in conducting Hg studies at the groundwater–lake interface is twofold: difficulties in collecting contamination-free samples at small (centimetres) intervals in sandy sediments and quantifying the natural variability in groundwater flow rates that exists over relatively small areas.

In this paper, we investigate the processes controlling the production and flux of MeHg at the littoral zone sediment–water interface of Pallette Lake, Wisconsin. We demonstrate a novel method for estimating net Hg methylation rates in sediments where MeHg profiles and groundwater flow rates have been measured and compare these rates with measured gross methylation rates determined by trace-level ^{203}Hg additions to intact incubated cores (Gilmour and Riedel 1995). Finally, we estimate MeHg production and flux in the context of other MeHg flux mechanisms for Pallette Lake.

Study area

Located in the Northern Highland Lake District of northern Wisconsin, Pallette Lake (46°04′N, 89°36′W) has no public vehicle access, and thus receives minimal human impact. It is a dimictic seepage lake; water enters by groundwater inflow and precipitation and leaves by groundwater outflow and evaporation. The lake has a surface area of about 0.70 km^2 and maximum and average depths of 19 and 10 m, respectively, and is situated in about 50 m of glacial outwash sediments that overlie Precambrian crystalline bedrock (Attig 1985). These deposits are primarily sand and gravel, which provide for effective hydraulic exchange between the groundwater system and lakes and streams. A neighboring lake 200 m to the east and about 2 m higher in elevation induces constant groundwater inflow along the northeast shoreline of the lake.

Materials and methods

Field sampling strategy

An established network of piezometers around the perimeter of Pallette Lake documented the existence of an inflow zone along the northeast shoreline and revealed that porewater chemistry in groundwater inflow zones (10% of the shoreline) and outflow zones differs markedly, including total Hg (Hg_T) (Krabbenhoft and Babiarz 1992). Our general strategy for estimating mass fluxes of Hg_T and MeHg from the littoral sediments to the lake was to simultaneously collect porewater chemical profiles and groundwater discharge data over the course of about 2 years (1992–1994). The observed chemical profiles in porewaters, then, are reflective of the balances between upward advective flow through the sediments and the biogeochemical processes controlling chemical constituent concentrations. For comparison with the mass flux estimates of MeHg, gross MeHg production within the sands was also estimated in situ using ^{203}Hg as a radiotracer. Methylation rate measurements were made in August 1993 at the lake's maximum temperature and in May 1994 about 2 weeks after ice-out. Concentrations of Hg_T and MeHg in sand cores were also measured at the same depth intervals that porewater and methylation rates were measured.

Estimating mass flux of Hg_T and MeHg

To calculate the mass loading rate of Hg_T or MeHg from groundwater discharge, we applied the following:

$$(1) \qquad M = (C_{PW})\,(q)\,(A)\,(t)$$

where M is the areal mass loading rate of Hg_T or MeHg. C_{PW} is the concentration of Hg_T or MeHg at the sampling interval closest to the interface (nanograms per litre), q is the groundwater flow rate (metres per day), A is the area of littoral zone with groundwater inflow (square metres), and t is time (days). Because we have never observed MeHg in groundwater at depths greater than about 0.5 m below the sediment–water interface, and we measure the vertical flow rate, this equation also provides an estimate of net MeHg production within littoral sediments. These estimates are compared against the ^{203}Hg-determined gross methylation rates.

Groundwater inflow measurements were made on 16 dates during ice-free periods from 1992 to 1994. On each occasion, two or three measurements where made in the same location as porewater sampling. Measurements were made using a variable-head infiltrometer, an adaptation of a constant-head permeameter (Means and Parcher 1963), to estimate the vertical hydraulic conductivity of the sediments (K_v, metres per day). The specific discharge rate, q, in eq. 1 is derived by multiplying K_v by the measured hydraulic gradient (metres per metre), which was determined by the use of minipiezometers that were installed at each site when groundwater flow rates were measured.

Porewater and sediment sampling methodologies

All sampling aspects of this research used ultraclean sampling and analytical techniques (Fitzgerald and Watras 1989). Samples were filtered using preashed, quartz-fiber filters (nominal 0.7-μm pore size). Hg_T samples were acidified to 1% HCl, while MeHg samples were put on dry ice in the field and kept frozen until analysis. Ancillary samples for pH, alkalinity, sulfate, and chloride were stored in polyethylene bottles, DOC and dissolved inorganic carbon (DIC) samples in glass vials, and CO_2 and methane samples in glass syringes. In a limited number of cases, samples were taken for oxygen-18 (^{18}O) analysis for determination of a porewater dilution factor.

In situ porewater samples were collected using two devices: one that used an applied vacuum and another that used free drainage by gravity. With either method, samples were taken at three to five depths between 0 and 10 cm.. The applied-vacuum method involved the use of a borosilicate-glass frit which was connected by Teflon tubing to an inline filter and then to a Teflon transfer jar to which a

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vacuum was applied. The frit was inserted vertically into the littoral zone sediments (about 0.2–0.5 m lake water depth) to the desired sampling depth and held in place. The only exception to this was when taking samples at the 1 cm depth interval, the frit was inserted at an acute angle to minimize leakage of lake water into the frit. A hand suction pump was used to apply a vacuum (about 40 kPa) to the transfer jar, which induced flow of porewater into the transfer jar. About 30–60 mL of sample was withdrawn for each insertion at a particular depth; this process was repeated three to five times at the same depth within about a 0.5-m quadrat to generate enough sample volume for all the analyses. The pooled sample from the transfer jar was then split among the various sample containers.

The free-drainage method for porewater sampling involved the use of a 30.5-cm-long, 2.54-cm (outside diameter) Teflon piezometer point, which has four slots (0.04 cm wide) over a 1-cm interval near its sharpened endpoint. The device was pushed into the littoral sediments to the desired depth and then evacuated using a Teflon sample line and a peristaltic pump. The pump was then set to a minimum speed so that the pumping rate approximately equaled the drain rate of porewater into the evacuated sampler.

Both porewater sampling methods described above are potentially susceptible to sample dilution by lake water in response to the imposed downward hydraulic gradient. This was especially true for the sampling intervals closest to the interface (0–2 cm). For temperate lake systems, stable isotopes of water (^{18}O and deuterium) can be used to detect the presence of lake water in porewater samples and to estimate the dilution factor for individual porewater samples (Krabbenhoft and Webster 1995). Here, we use a separation quotient similar to that of Krabbenhoft et al. (1990):

$$(2) \qquad \frac{\delta^{18}O_L - \delta^{18}O_S}{\delta^{18}O_L - \delta^{18}O_{GW}} = X$$

where X is the fraction of groundwater in the porewater sample and $\delta^{18}O$ are the ^{18}O results expressed in per mil (‰) notation relative to V-SMOW for lake water (L), groundwater (GW), and the porewater sample (S). By obtaining the dilution factor, and the concentration of Hg (Hg_T or MeHg, nanograms per litre) in the sample and lake water, we can estimate the Hg concentrations in those samples where dilution may be significant using the following:

$$(3) \qquad X[{}^*Hg_S] + (1 - X)\,[Hg_L] = Hg_{obs}$$

where Hg_{obs} and Hg_L are the concentrations of Hg_T and MeHg in the analyzed porewater and lake water samples, respectively, and $^*Hg_S$ is the estimated dilution-corrected concentration of Hg_T or MeHg at the sampling interval of interest.

Intact sediment cores for the ^{203}Hg-determined gross methylation rates were sampled by hand into acid-leached 4.8-cm-diameter PVC tubes. Sediments were extruded and sectioned within a few hours of collection. For each sampling date and site, one or two cores were sectioned into 2- to 4-cm segments with depth to about 10 cm, plus three 0- to 4-cm sections were collected from separate cores. All sediment samples were immediately frozen.

Analytical methods

Hg_T in aqueous samples was determined by cold-vapor atomic fluorescence spectroscopy (CVAFS) following oxidation with BrCl, reduction by $SnCl_2$, and purge and trap of the evolved Hg^0 onto gold-coated sand columns (Gill and Fitzgerald 1987; Bloom and Fitzgerald 1988). MeHg was analyzed using the distillation and aqueous-phase ethylation method of Horvat et al. (1993) and detection by CVAFS. Quantification limits for Hg_T and MeHg for surface waters are about 0.06 and 0.02 ng/L, respectively; porewater samples have higher quantification limits, about 0.4 ng Hg_T/L and 0.1 ng MeHg/L, due to their inherently smaller volumes. Solid-phase MeHg and Hg_T analyses were performed as described above. Modifications, SRM analyses, and spike recoveries are described in Gilmour and Riedel (1995).

Ancillary parameters were determined by the following methods: alkalinity by potentiometric Gran titration, sulfate and chloride by high-performance liquid chromatography, DIC and DOC by a carbon analyzer that uses acidification and persulfate–UV oxidation, and methane and CO_2 by gas chromatography with an inline methanizer and flame ionization detection. Reduced sulfur concentrations were measured by cold-acid distillation (AVS, acid-volatile sulfides) and chromium reduction (CRS, chromium-reducible sulfides) (Fossing and Jorgensen 1989). Dissolved sulfide in porewaters was preserved in sulfide antioxidant buffer (Brouwer and Murphy 1994) and measured electrochemically using a selective ion probe.

Gross MeHg production from inorganic Hg(II) within sediments was estimated using custom-synthesized high specific activity ^{203}Hg as a radiotracer, as described in Gilmour and Riedel (1995). The goal of this method is to use ^{203}Hg of specific activity sufficient to act as a tracer in natural sediments and waters. In short-term experiments, this should provide gross methylation rates (Xun et al. 1987). Rates estimated using greatly elevated Hg concentrations may not reflect rates at ambient Hg concentrations and speciation (Gilmour et al. 1991). Since the specific activity of commercially available ^{203}Hg is not sufficient to be used as a tracer in natural waters or in uncontaminated sediments, high specific activity $^{203}HgCl_2$ was produced for this work by custom synthesis from ^{202}Hg. Two batches were used in this work, with specific activities of 1.03×10^9 Bq/mg (August 1993) and 6.18×10^8 Bq/mg (May 1994).

Methylation within sediments was estimated by injection of $^{203}HgCl_2$ into intact sediment cores at 1 cm depth intervals. The $^{203}HgCl_2$ solution was preincubated with sediment porewaters for an hour before injection into cores to allow formation of dissolved Hg complexes. Sealed cores were incubated in situ for a period of hours to 2 days and then cut with depth into 2.5-cm slices and immediately frozen. Injections of ^{203}Hg were generally made to 10 cm depth. Information on the time course and substrate concentration dependence of $Me^{203}Hg$ production in these sediments is given in Gilmour and Riedel (1995).

$Me^{203}Hg$ was extracted from aliquots of thawed sediment, as described in Gilmour and Riedel (1995), and assayed using a 3-in. (7.6-cm) NaI-well gamma detector. The ambient methylation rate was calculated by multiplying the fraction of added ^{203}Hg methylated per day by the ambient Hg_T concentration in the horizon of interest. This calculation assumes that ^{203}Hg is added as a tracer and that the speciation and sediment–water partitioning of added Hg mimic those of ambient Hg. Concentrations of Hg_T in Pallette inflow sands average about 1 ng/g (dry weight), and our additions increased the Hg_T concentrations by 2.7 ng/g (August 1993) and 0.8 ng/g (May 1994).

Results and discussion

Porewater profiles

Porewater samples were acquired from the center of the groundwater inflow zone to avoid sampling in areas where the direction of flow may reverse occasionally due to transient hydrologic conditions. On three occasions, we sampled using both the applied-vacuum and gravity-drain methods to test the efficacy of these methods for sampling in sandy littoral sediments. The results of each method show similar overall concentration ranges for Hg_T, as well as similarly shaped profiles with subsurface maxima (Fig. 1).

^{18}O analytical results show that the subsurface maxima in the porewater profiles are due to sample dilution by lake water. During each sampling period, and for both sampling methods, the deepest porewater samples have $\delta^{18}O$ values very similar to those observed previously (range −11.0 to −11.7‰) for groundwater at Pallette Lake (Krabbenhoft and Babiarz 1992) and are very distinct from lake water which averages about

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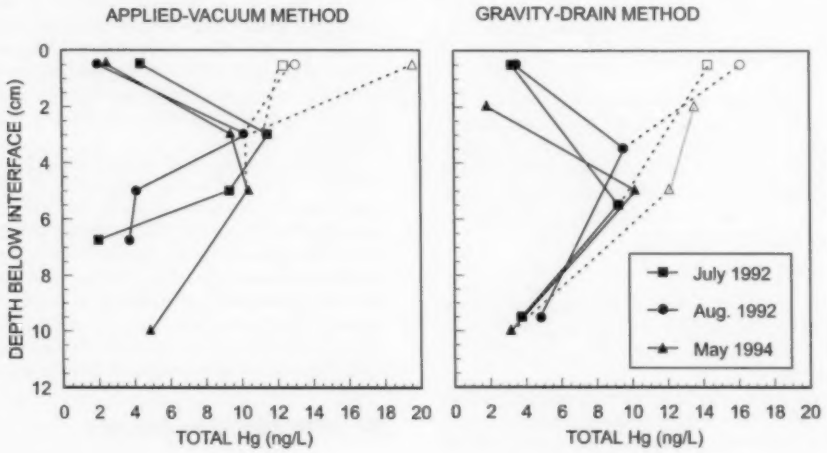
Table 1. Results of Hg_T and ^{18}O analyses on porewater samples, and calculated values of groundwater fraction and dilution-corrected Hg_T in near-surface porewater samples.

Method	Date	Depth (cm)	$\delta^{18}O$	GW fraction[a]	Hg_T obs. (ng/L)	Hg_T calc. (ng/L)[b]
Applied vacuum	July 1, 1992	0.5	–6.07	0.30	4.42	12.4
	Aug. 24, 1992	0.5	–4.39	0.08	1.96	13.0
	May 9, 1994	0.5	–4.76	0.08	2.49	19.6
	May 9, 1994	3.0	–10.52	0.89	9.45	10.5
	May 9, 1994	5.0	–11.44	1.02	10.37	10.2
	May 9, 1994	6.75	–11.58	1.04	4.97	4.81
Gravity drain	July 1, 1992	0.5	–5.14	0.17	3.26	14.3
	July 1, 1992	10.0	–11.10	1.00	3.81	3.81
	Aug. 24, 1992	0.5	–4.96	0.16	3.43	16.1
	Aug. 24, 1992	6.75	–11.01	1.00	4.85	4.85
	May 9, 1994	2.0	–4.70	0.07	1.88	13.5
	May 9, 1994	5.0	–10.09	0.83	10.25	12.1
	May 9, 1994	10.0	–11.21	0.99	3.21	3.23
	May 9, 1994	91	–11.30	1.00	na	na

[a]Calculated groundwater fraction using eq. 2 and the $\delta^{18}O$ results for the deepest sampling interval for a given date and measured lake water $\delta^{18}O$ values of –3.92, 3.82, and –4.20‰ on July 1, 1992, August 24, 1992, and May 9, 1994, respectively.
[b]Estimated porewater Hg_T concentration using eq. 3.

Fig. 1. Comparison of sample collection methods for Hg_T porewater profiles from the inflow zone. Solid symbols show the actual sample Hg_T concentration; open symbols show the corrected Hg_T concentration after the results of the ^{18}O analysis were used in eqs. 3 and 4 to correct for lake water dilution.



–4.0‰. If we assume conservative mixing of a two-endmember system (lake and groundwater), we can calculate the fraction of groundwater present in our samples (Table 1). These calculations show that shallow porewater samples (<2 cm depth) are dominantly lake water (70–93% lake water), but that the samples from about 3 cm depth or more are representative of porewater (83–100% groundwater). By using the average lake water concentration of 1 ng Hg_T/L and the calculated lake water fraction for the shallow samples, corrected Hg_T concentrations were derived. The corrected Hg_T values show a more linear trend with the samples collected at depth, especially the profiles from the gravity-drain method (Fig. 1). The corrected Hg_T concentrations at 1 cm depth in this figure are very similar to the values observed by Krabbenhoft and Babiarz (1992), who also sampled porewaters from the littoral zone of Pallette Lake, but extracted porewaters from intact sediment cores using suction. That method is considerably more laborious but not as susceptible to sample dilution by lake water. Based on these results, we conclude that subsurface maxima in Hg_T concentration are the result of a sampling artifact and that concentrations of Hg_T actually increase nearly linearly toward the sediment–water interface.

Porewater samples for MeHg analysis were taken using the applied-vacuum method on seven occasions from May 1993 to May 1994 (Fig. 2). Three observations can be made from these data: (*i*) many samples fall at or below the quantification limit of 0.1 ng MeHg/L, (*ii*) those profiles where MeHg is above the quantification limit generally show subsurface maxima, and (*iii*) those profiles where MeHg is appreciably above the quantification limit were all sampled in spring and early summer.

On three occasions (June 1993, August 1993, and May

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Fig. 2. Porewater MeHg profiles from the inflow zone at Pallette Lake using the applied-vacuum method for sampling. Solid symbols show the actual sample MeHg concentration; open symbols show the corrected MeHg concentration after the results of the ^{18}O analysis were used in eqs. 2 and 3 to correct for lake water dilution.

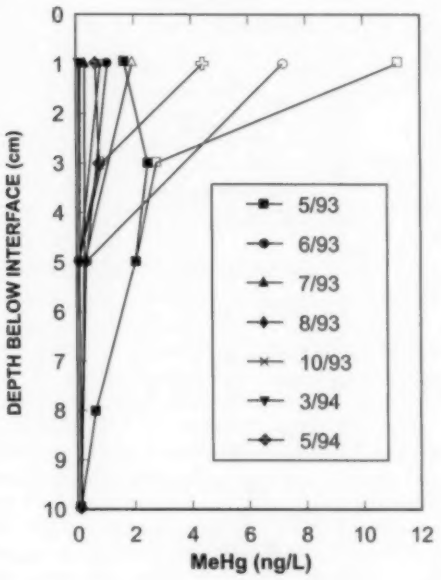
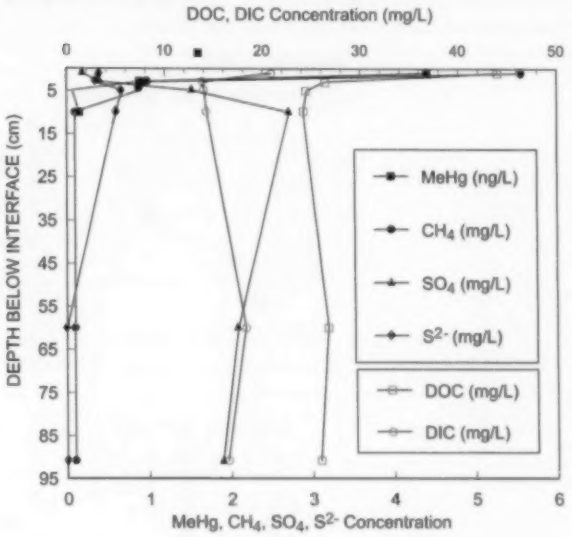


Fig. 3. Ancillary porewater chemistry data for samples collected in May 1994 from the groundwater inflow zone. CH_4, methane; SO_4, sulfate; S^{2-}, sulfide.



1994), we used a 15-mL portion of the porewater samples from 1 and 3 cm depth for ^{18}O analysis and found similar dilution ratios as those presented above. The three samples taken at 1 cm depth averaged 5.05‰ (range –4.76 to –5.65 ‰) whereas the three samples from 3 cm depth averaged –10.31‰ (range –10.22 to –10.64‰). Lake and groundwater samples were not taken for ^{18}O analysis in June 1993 and August 1993 Considering that these two endmember waters show very little variability in ^{18}O results, we calculated the fraction of groundwater in these samples using the observed averages of –3.98 and –11.1‰ for lake and groundwater, respectively. Using these data and eq. 4, we corrected MeHg concentrations at the 1 and 3 cm depths (Fig. 2). The corrected profiles show that the greatest MeHg concentrations occur at the shallowest sampling depth (1 cm), and the greatest values are observed in spring and early summer. The porewater samples with high MeHg concentrations (>2 ng/L) all show a high proportion of Hg_T as MeHg, ranging from about 30 to 50%, which is reflective of rapid rates of Hg methylation in littoral sediments.

Ancillary measurements on porewater samples demonstrated the dynamic character of biogeochemical processes near the sediment–water interface and the fine scale (centimetres) at which large changes in critical constituents occur (Fig. 3). These data were also corrected for lake water dilution. The most rapid rate of change for all the constituents occurs in the upper 10 cm. Between 60 and 90 cm depth, sulfate, sulfide, methane, DOC, and DIC show little or no change, and, with the exception of sulfide, only modest changes were observed between 10 and 60 cm depth. About 98% of the sulfate advecting upward is lost between 0 and 10 cm. Sulfide concentrations increase from less than detectable at 60 cm to a subsurface maximum of 0.8 mg/L at 5 cm. The decrease in porewater sulfide at depths less than 5 cm may be due to precipitation with iron, as iron hydroxide is visible in the near-surface sediments, or less sulfide is formed above 5 cm depth. In littoral sediments of the groundwater inflow zone, sulfate reduction is driven by groundwater sulfate advecting upward through sediments. These concentration profiles of major constituents are reversed from patterns seen in nonadvecting areas or where lake water and transport downward is the ion source (Rudd et al. 1986). Methane, DIC, and DOC ali show dramatic increases over the upper few centimetres. During three other sampling periods (including March 1994 when the lake was ice covered) the same suite of ancillary parameters were measured and similar profiles were observed, suggesting that these processes operate continuously.

Details of biogeochemical processes in the top 10 cm, where microbial activities are highest, are shown in Fig. 4 for August 1993 and May 1994, within 2 weeks of ice-out. Gross MeHg production, measured using ^{203}Hg as a tracer, was maximal at or near the sediment surface. In both spring and summer, sulfate was depleted as groundwater was advected toward the sediment surface, and sulfate reduction rates decreased concomitantly. Sulfate reduction rates were about twice as high in August as in April. Solid-phase sulfur (AVS and CRS) accumulated over the summer, and dissolved sulfide increased somewhat as well. However, the low sulfate concentrations in Pallette Lake and the low organic content of these sandy littoral sediments are reflected in overall very low sulfide concentrations in all phases. Hg methylation rates were maximal just above the zone of maximum sulfate reduction in both May and August. While bulk and dissolved Hg_T concentrations remained similar, MeHg accumulated in bulk-phase sediments from May to August. However, porewater MeHg concentrations were much higher in the spring, as discussed above.

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Fig 4. Sediment depth profiles of Hg methylation rates and sulfur and Hg chemistry in Pallette Lake littoral sediments in the groundwater inflow zone for May 9, 1994, and August 26, 1993. Hg methylation rates were measured using ^{203}Hg as a radiotracer and calculated using total sediment Hg as substrate. Each profile represents an individual sediment core. AVS (acid-volatile sulfides) and CRS (chromium-reducible sulfides) profiles shown are averages of at least three cores, with standard deviations shown as error bars. Sulfate, sulfide, Hg, and MeHg in porewaters were taken from pooled replicate samples, and sulfate concentrations are shown for above the sediment–water interface.

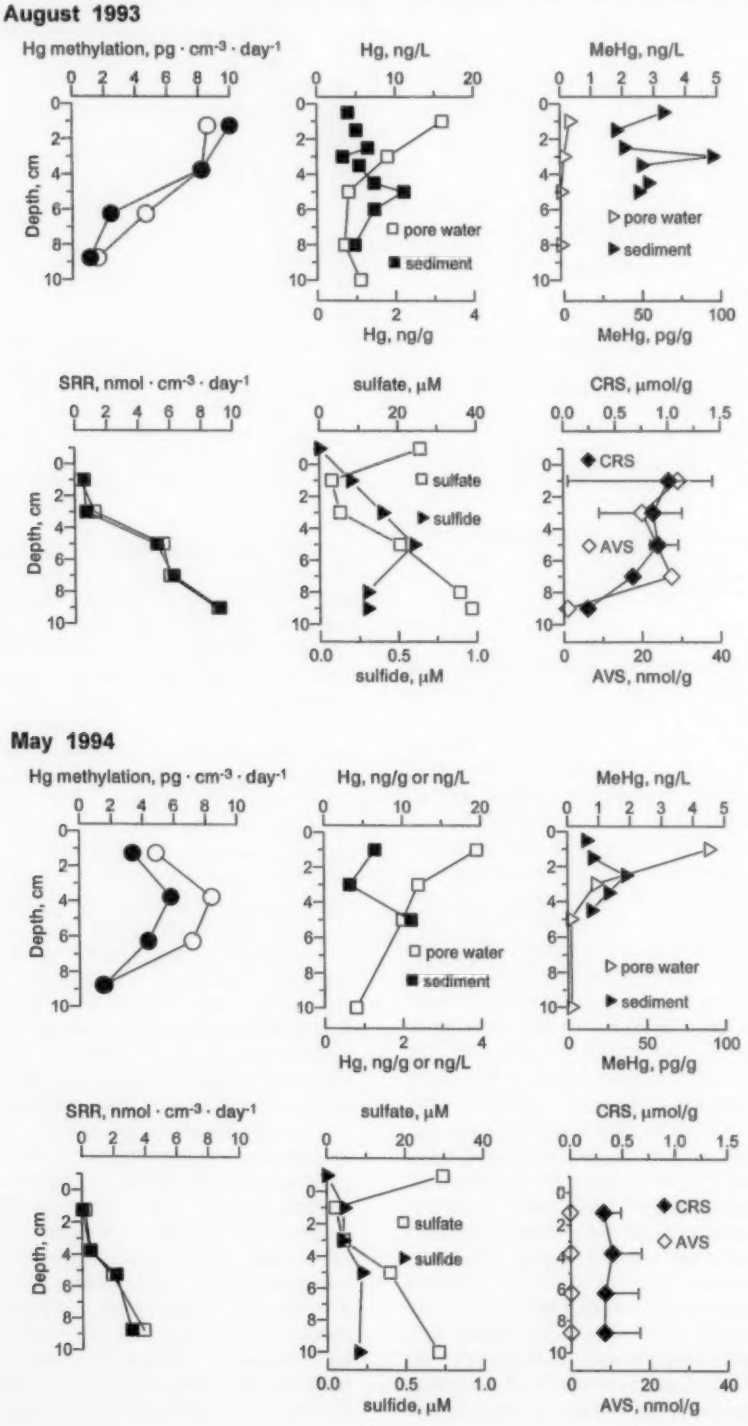

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Table 2. Calculated seasonal mass-flux rates of MeHg and Hg_T for Pallette Lake.

	Flow rate (m^3/day)	Days	Concentration range (ng/L)	Mass flux ($ng \cdot m^{-2} \cdot day^{-1}$)	Whole-lake flux (mg)
MeHg					
Springtime	196	76	0.8–11.2	4.5–61.7	12–164
Summer–fall	126	130	0.0–1.9	0–7.0	0–32
Total for ice-free period (mid-April to early November)					12–196
Hg_T					
Springtime	196	76	12.4–19.6	69–109	184–292
Summer–fall	126	130	12.4–19.6	44–71	203–321
Winter	126	159	12.4–19.6	45–70	248–392
Total for ice-free period (mid-April to early November)					387–613
Annual flux					635–1005

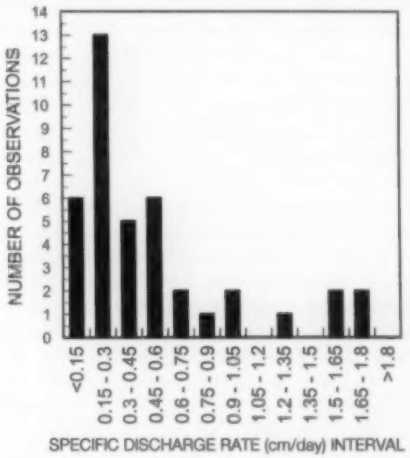
Mass flux of Hg_T and MeHg to Pallette Lake

Groundwater discharge rates at Pallette Lake show a lognormal distribution (Fig. 5), a common observation for natural sediments. The average discharge rate of all 40 measurements was 0.48 ± 0.42 cm/day, but springtime measurements were greater than those made in summer and fall: 0.56 ± 0.59 cm/day(April–June, $N = 21$) versus 0.36 ± 0.34 cm/day (July–November, $N = 19$). Given that the area of lakebed contributing groundwater discharge to Pallette Lake was essentially constant during this study (3.5×10^4 m^2), we can convert these seasonal vertical discharge rates to volumetric flow rates of 196 m^3/day (springtime) and 126 m^3/day (summer–fall). This seasonal difference is due to greater hydraulic gradients in spring, resulting from recharge of melting snow and rainy periods during spring and early summer.

Using the volumetric flow rates described above and the corrected Hg_T and MeHg concentrations from Table 1 and eq. 1, mass flux rates for the ice-free period at Pallette Lake were estimated (Table 2). Because MeHg porewater data and groundwater discharge rates show significant differences between the spring and summer–fall periods, we calculated MeHg flux rates for both periods. These calculations show that the majority of MeHg advected from the littoral zone sediments at Pallette Lake occurs in the spring; the average rate for spring is 11.1 $ng \cdot m^{-2} \cdot day^{-1}$ (range 1.1–23.5) compared with 0.5 $ng \cdot m^{-2} \cdot day^{-1}$ (range 0–1.4) for summer–fall. For the 76-day spring period, this equates to a production rate of 3–62 mg MeHg for the groundwater inflow area compared with 0–6 mg MeHg for the 130-day summer–fall period. If we had based our calculations on a diffusive transport model, and neglected advection, the estimated MeHg flux from the littoral zone would be insignificant.

The large range in calculated MeHg flux estimates (12–196 mg over the ice-free period) is reflective of the observed range of MeHg concentrations in porewaters and the variability in flow rates. When compared with the estimated atmospheric MeHg deposition rate to Pallette Lake of 49 mg/year (Lamborg et al. 1995), the efflux from littoral sediments can be an important (or dominant) source of MeHg to Pallette Lake. This is especially true in early spring when up to 164 mg of MeHg effluxes from the littoral sediments and about 15 mg is contributed by atmospheric deposition. The springtime efflux rate of MeHg (4.5–61.7 $ng \cdot m^{-2} \cdot day^{-1}$) is as much as three times the estimated rate for the water column during a stratified period when sulfate reduction was occurring (10–20 $ng \cdot m^{-2} \cdot day^{-1}$, Watras et al. 1995).

Fig. 5. Groundwater discharge velocities measured from the groundwater inflow zone using the infiltrometer test method.



For Hg_T, we did not have enough data to detect seasonal differences in the observed range of Hg_T concentrations at the top of individual profiles, and the data we have are not as disparate as the MeHg profiles. Thus, we use only one concentration range in our flux estimates (Table 2). These calculations show that about 387–613 mg Hg_T is contributed to Pallette Lake during the ice-free period. Of this Hg_T flux, 2–50% is MeHg. If we extend these calculations by assuming that the summer–fall flow and concentration data are representative of winter conditions, an estimated annual littoral zone Hg_T flux of about 600–1000 mg is derived. This range of Hg_T flux values compares favorably with the estimate of Krabbenhoft and Babiarz (1992), who used different methods for acquiring porewater samples and estimating the groundwater flux rate, and estimated an annual flux rate of 700 mg. About 30–40% of this Hg flux is new Hg from groundwater flow; the remainder is presumably recycled Hg from the epilimnion (including MeHg) brought to littoral sediments by particle settling and released to porewaters by mineralization. When compared with the estimated annual atmospheric flux of about

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Table 3. Gross MeHg production rates estimated from ^{203}Hg methylation, and advective MeHg efflux from sediments.

	Gross MeHg production ($ng \cdot m^{-2} \cdot day^{-1}$)					
	Based on solid-phase Hg		Based on porewater Hg			MeHg advection
Date	Average	SD	Average	SD	*N*	($ng \cdot m^{-2} \cdot day^{-1}$)
Aug. 1993	596	55	0.91	0.25	6	0.58
May 1994	449	84	1.00	0.20	4	46.6

7000 mg, we conclude that, unlike the MeHg flux estimates, the littoral zone sediments are not a dominant Hg_T source.

MeHg production rates

To estimate MeHg production using the tracer-level ^{203}Hg method, the specific activity of the Hg available for methylation must be known. The pool of Hg available to microbes for methylation in natural sediments is presently unknown, however, and remains a key to understanding and quantifying in situ methylation rates. We used two methods to calculate specific activity in sediments. Gross MeHg production from ^{203}Hg methylation was calculated by multiplying the methylated fraction of the added ^{203}Hg by either the concentration of solid-phase Hg_T or the porewater Hg_T concentration in sediments on the sample date (Table 3). Using solid-phase Hg plus added ^{203}Hg as the available Hg pool gives methylation rates that are between two and three orders of magnitude higher than rates derived using porewater Hg as the sole substrate. The time required to generate the observed MeHg pool in the littoral sediments would be on the order of 10–30 days using the methylation rate estimated with solid-phase Hg_T as substrate (Table 3). However, using the porewater-based calculation, turnover times would be decades. MeHg production using porewater Hg_T concentration was insufficient to support MeHg advection in May but not in August (Table 4). This calculation implies that some of the solid-phase Hg is available for methylation, either directly or through rapid dissolution. However, methylation rates estimated using the entire solid-phase pool exceeded the estimated MeHg advection.

Sources and sinks for MeHg in the groundwater inflow sands

The groundwater inflow zone at Pallette Lake offered a unique opportunity to make independent estimates of MeHg production. High MeHg concentrations and a steep gradient of MeHg in porewaters suggested that MeHg production was fairly rapid in these sediments. Low organic carbon content of the sands results in a relatively low affinity of Hg and MeHg for solids. As a result, porewater is a more important component of the Hg budget in this location than for most sediments. Because input, advection, and accumulation of MeHg could be directly measured, a budget could be constructed allowing independent estimation of MeHg production (Table 4). The budget could then be used to constrain our estimates of the Hg(II) substrate pool for methylation.

Advection is not the only potential sink for MeHg produced within the littoral sediments at Pallette Lake. The mass of MeHg in the groundwater inflow area sands increased over the course of the summer, but Hg_T did not. The increase in MeHg

Table 4. Budget for MeHg in Pallette Lake sediments in the groundwater inflow zone, 0–10 cm depth.

	May	August
Sources ($ng \cdot m^{-2} \cdot day^{-1}$)		
Gross MeHg production, ^{203}Hg based	449	596
Sinks ($ng \cdot m^{-2} \cdot day^{-1}$)		
Buildup of MeHg in solid phase (July–Aug. 1992)	187	187
Buildup of Hg in solid phase	0	0
MeHg advection	47	1
Sum of MeHg loss terms	234	188
MeHg pool size (ng/m^2)	3000	10 200

in bulk sediment was estimated from the increase in the average concentration in the top 10 cm during July–August 1992, when the most intensive sampling of sediments was performed. Average MeHg concentrations increased from 0.16 to 0.57 ng/g. This in situ accumulation of MeHg appears to be a larger sink for MeHg produced in the littoral sediments than advective transport to the overlying lake (by a factor of 10–100). The sum of accumulation and advection represents a lower bound on net MeHg production, assuming that deposition of particulate MeHg to littoral sediments is insignificant. Demethylation is also a potentially important loss term for MeHg, but this process was not measured directly in this study. Although the MeHg sediment accumulation data were only collected during summer, we assume here that the estimated accumulation rates are applicable to the spring as well.

For both May and August, the sum of the MeHg advection and solid-phase accumulation equaled one third to one half of the gross MeHg production rate estimated using ^{203}Hg addition (Table 4). Advection of MeHg from sediments appears to account for a small percentage of gross methylation at this site, especially in summer when flow rates are lower. Based on this rough budget, our estimates of gross MeHg production made using near-tracer-level ^{203}Hg addition appear to overestimate net methylation rates by less than a factor of 2, although there are significant uncertainties in the budget.

We have also examined the accuracy of the radiotracer method by comparison with sediment incubation methods (Gilmour and Riedel 1995) using no added Hg. In Pallette Lake littoral sediments outside the inflow zone, the incubation method yielded 90 $ng \cdot m^{-2} \cdot day^{-1}$ in June 1992 whereas the ^{203}Hg method yielded 350 $ng \cdot m^{-2} \cdot day^{-1}$ in May 1994. We also found reasonable agreement between these methods in another Wisconsin Lake (Gilmour and Riedel 1995). Taken together, these comparisons suggest that a substantial fraction of solid-phase Hg is available for methylation and that use of tracer additions of ^{203}Hg to sediments provides a reasonable estimate of gross in situ methylation rates.

Seasonality of MeHg production and flux

The general patterns of gross MeHg production and MeHg efflux from sediments were not the same, nor was the change in the magnitude of each of these processes across seasons. Gross MeHg production was only slightly higher in August at 25°C than in May at 8°C. We speculate that the rapid resupply of microbially available substrates, including Hg, might account for the relatively high rates of methylation observed in spring. However, advection of MeHg from sediments was

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about 100 times higher in spring than in summer. MeHg concentrations in porewaters in the summer were quite low. If gross MeHg production rates are relatively constant over the ice-free season, much lower MeHg concentrations in summer might be attributed to increased rates of demethylation, or a change in the partition coefficient for MeHg onto solids. Other researchers using nontracer methods (Xun et al. 1987) have observed that demethylation rates in epilimnetic sediments are highest in spring to early summer, while the methylation/demethylation ratio is highest in late summer at maximum sediment temperature (Korthals and Winfrey 1987; Ramlal et al. 1993). The discrepancy between gross MeHg production and MeHg advection is more likely explained by increased partitioning of MeHg onto the solid phase. MeHg partition coefficients are low in these sands compared with organic sediments, about 100–1000 $L \cdot kg^{-1}$. Partition coefficients for both Hg and MeHg were lowest in surface sediments and were lowest in the spring. In Pallette Lake sands, the concentration of solid reduced sulfides (a product of microbial sulfide reduction) does increase over the summer (Fig. 4), possibly providing a larger surface area and possibly more ligands for MeHg binding. However, the relative importance of kinetic and thermodynamic control over porewater Hg and MeHg concentrations is not known.

Acknowledgments

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Dispersal of male and female Antarctic fur seals (*Arctocephalus gazella*)

I.L. Boyd, D.J. McCafferty, K. Reid, R. Taylor, and T.R. Walker

Abstract: This study examined the foraging locations of adult male and female Antarctic fur seals (*Arctocephalus gazella*) in the Scotia Sea during the postbreeding period. Satellite transmitters were used to track adult males and females and to obtain information about dive depths. Male fur seals migrated away from the breeding area during the postbreeding period whereas females remained close to the breeding grounds and foraged in the same area during two consecutive years. The most intensive foraging by females was associated with the edge of the continental shelf of South Georgia. Males dived deeper than females. Counts of males at South Georgia and at the South Orkney Islands support the result from satellite tracking data showing that males move from South Georgia to the South Orkney Islands at the end of the breeding season. Unlike males, females were limited in their foraging range by the necessity to return to feed dependent young, so breeding sites are likely to be located close to foraging areas that are optimal for females. Locations used for feeding by females were avoided by males, either because they were suboptimal for males or because foraging by females at South Georgia causes local depletion of food, and males, which have the option to forage further afield, can forage more successfully in regions where there are no females. Comparison with fisheries data also suggests that these fur seals are targeting the most abundant exploitable prey.

Résumé : Cette étude porte sur les lieux d'alimentation des otaries des Kerguelen (*Arctocephalus gazella*) adultes, mâles et femelles, dans la mer de Scotia après la période de reproduction. On a utilisé des émetteurs satellitaires pour pister des mâles et des femelles adultes et pour obtenir de l'information sur leur profondeur de plongée. Les otaries mâles se sont éloignées de l'aire de reproduction après l'accouplement tandis que les femelles sont demeurées à proximité et se sont alimentées dans la même région durant deux années consécutives. C'est à la bordure de la plate-forme continentale de l'île South Georgia que les femelles se sont alimentées le plus intensivement. Les mâles plongeaient plus profondément que les femelles. Les dénombrements des mâles à l'île South Georgia et aux îles South Orkney confirment les résultats du pistage satellitaire qui montre que les mâles passent de l'île South Georgia aux îles South Orkney à la fin de la période de reproduction. À la différence des mâles, les femelles sont limitées quant à leur aire d'alimentation du fait qu'elles doivent retrouver leurs petits pour les nourrir, de sorte que les sites de reproduction doivent probablement se trouver près des aires d'alimentation qui sont optimales pour les femelles. Les lieux d'alimentation des femelles étaient évités par les mâles, soit qu'ils étaient sous-optimaux pour ces derniers, soit parce qu'en s'alimentant à l'île South Georgia, les femelles réduisent localement les ressources alimentaires, et les mâles, qui peuvent aller s'alimenter à distance des aires de reproduction, peuvent mieux se nourrir dans des régions exemptes de femelles. La comparaison avec les données sur les pêches laisse aussi penser que ces otaries recherchent la proie exploitable la plus abondante.
[Traduit par la Rédaction]

Introduction

The impact of predator populations on their prey has potential significance for the management of marine resources (Larkin 1996). Until recently, it has been difficult to estimate the spatial impact of predators on resources, even though reasonable estimates of the total impact of a predator population have been made (e.g., Lavigne et al. 1985; Butterworth et al. 1995; Shelton et al. 1995; Guinet et al. 1996). The introduction of methods of tracking seabirds (Jouventin and Weimerskirch 1990; Prince et al. 1997) and marine mammals (e.g., Martin et al. 1993; McConnell and Fedak 1996; Stewart et al. 1996) using satellite transmitters has begun to provide the level of information necessary to determine spatial patterns of impact. This study was designed as a first step towards estimating the dispersal of a marine mammal population that could eventually lead to estimates being made of the spatial impact it has on its prey.

In addition, dispersal patterns of marine top predators may be used to infer important variation in the ecology and vulnerability of metapopulations or of the different sex and age classes. For example, among mammals, sexual dimorphism of body size is most evident in marine species, and this may lead to differences in the ecology of the two sexes. Adult male sperm whales (*Physeter macrocephalus*) are three times the mass of adult females and there is a strong effect of latitude on the sex ratio of the population, with the largest males being found at the highest latitudes (Best 1979). Male elephant seals (*Mirounga* sp.), which are three to six times larger than females, also forage in different areas than females (Hindell et al. 1991; LeBoeuf et al. 1993; Stewart and DeLong 1993).

In this study, we examine the spatial distribution of male and female Antarctic fur seals (*Arctocephalus gazella*) during the postbreeding period (January–March) when both sexes

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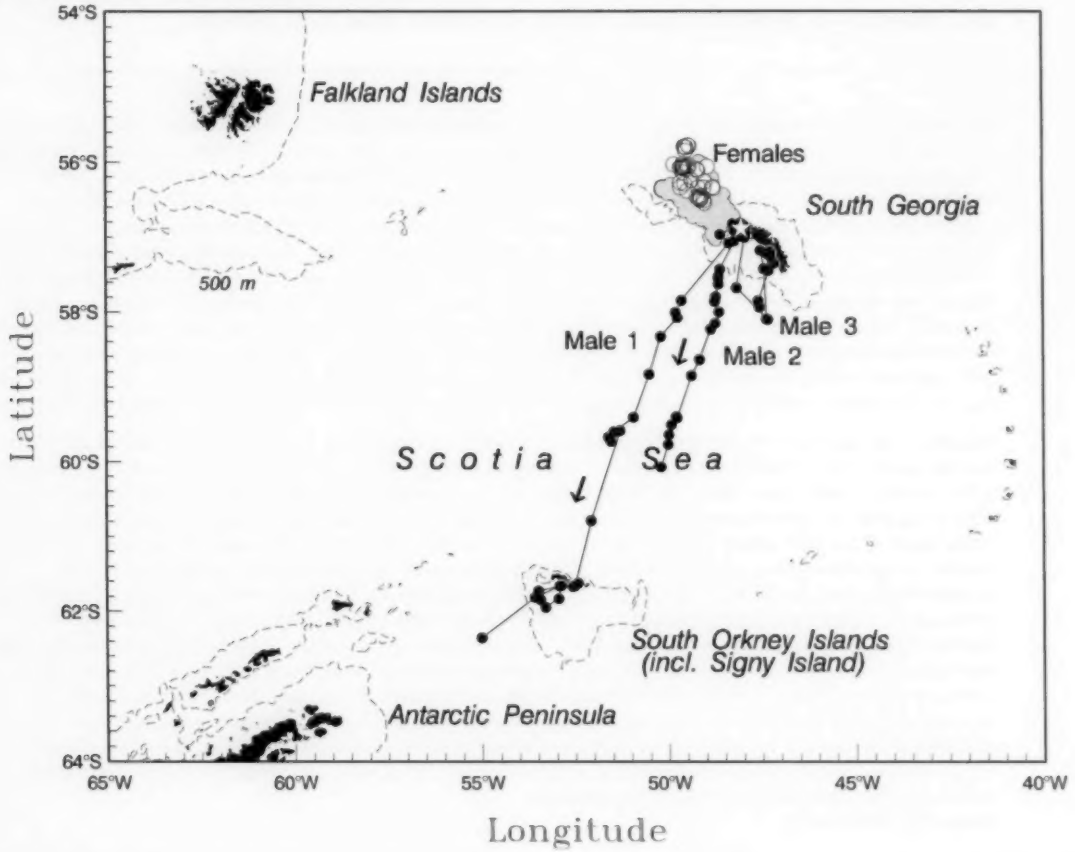
Fig. 1. Study region and geographic distribution of locations of Antarctic fur seals obtained from PTTs carried by each animal. Open circles are the locations of females in 1994–1995, the grey area is the location of females in 1995–1996, and solid circles are the locations of males in 1992–1993. The location of Bird Island, where the transmitters were deployed, is shown by the white star. The tracks of each male are shown individually and each position is linked by a line whereas the tracks of females are not shown individually. Arrows indicate the direction of movement of males 1 and 2. The 500-m isobath is shown.



depend mainly on a diet of krill (*Euphausia superba*) with a smaller proportion of fish (Daneri and Coria 1992; Reid and Arnould 1996). At this time, adult males, which are up to six times the mass of adult females, require food to recover from the mass lost during the breeding season (Boyd and Duck 1991), while most adult females are foraging to sustain themselves and a dependent pup (Boyd et al. 1991; Arnould et al. 1996*b*). Therefore, there is likely to be a high predator demand through this period. Moreover, the foraging range of lactating females is presumed to be restricted by the requirement to return to the pup, which remains ashore throughout the 4 months of lactation.

There is little information about the dispersion patterns of marine mammals while foraging at sea. In cases where marine mammals interact with commercial fisheries, there is a need to understand the spatial impact and distribution of these predators. Therefore, this study examined the hypothesis that (*i*) geographic distribution and dive depths should differ between the sexes in a marine mammal that has sexual dimorphism of body size and (*ii*) using current instrumentation, it is possible to measure the relative impact of a marine mammal predator at a spatial and temporal scale that will be useful to fisheries management.

Materials and methods

Study area

The study was centred on two specific locations in the region of the Scotia Sea: Bird Island (South Georgia) and Signy Island (South Orkney Islands) (Fig. 1). The southern Scotia Sea is covered seasonally by pack ice that occasionally extends as far north as South Georgia in late winter.

Daily counts of the total number of adult male Antarctic fur seals were made at specific locations at Bird Island and Signy Island to document seasonal abundance patterns (Boyd 1989). These locations were consistent across years (1992–1995 at Bird Island and 1992–1994 at Signy Island). At Bird Island, counts were made between 1 November and 10 January and these covered the breeding season. Relatively small numbers of males are found at Bird Island, and South Georgia in general, at other times of year (Boyd 1989). At Signy Island, counts began on 1 January and continued until the sea froze, usually during June. No counts were made before 1 January because fur seals are not normally present before this date.

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Table 1. Deployments of PTTs on adult male and female Antarctic fur seals from Bird Island during the austral summers of 1992–1993, 1994–1995, and 1995–1996.

Tag No.	Year	Sex	Number of locations[a]	Start date of deployment	Duration of deployment (days)	Seal mass (kg)
w3815 (male 1)	1993–1994	Male	43	19 Dec. 1992	50	159.0
w3817 (male 2)	1993–1994	Male	61	21 Dec. 1992	22	145.0
w3811 (male 3)	1993–1994	Male	58	19 Dec. 1992	29	122.5
w2403	1994–1995	Female	2	27 Dec. 1994	7	33.0
w2943	1994–1995	Female	9	5 Feb. 1995	9	40.0
w4687	1994–1995	Female	7	13 Jan. 1995	9	31.5
w5201	1994–1995	Female	5	4 Dec. 1995	5	38.5
w5319	1994–1995	Female	21	23 Jan. 1995	7	41.5
w1639	1995–1996	Female	15	29 Jan. 1996	17	40.5
w1753	1995–1996	Female	26	21 Jan. 1996	15	38.5
w2385	1995–1996	Female	33	26 Jan. 1996	17	46.0
w2935	1995–1996	Female	4	7 Jan. 1996	6	39.0
w3805	1995–1996	Female	5	19 Feb. 1996	13	37.5
w3847	1995–1996	Female	2	1 Feb. 1996	17	43.5
w4605	1995–1996	Female	24	10 Feb. 1996	17	38.5
w4633	1995–1996	Female	39	19 Feb. 1996	18	47.0
w5149	1995–1996	Female	3	7 Dec. 1995	7	46.0
w5344	1995–1996	Female	10	8 Dec. 1995	8	34.5
w5346	1995–1996	Female	1	15 Dec. 1995	14	41.5
w5347	1995–1996	Female	3	17 Dec. 1995	9	42.0
w5350	1995–1996	Female	42	5 Jan. 1996	19	41.5
w5351	1995–1996	Female	7	5 Jan. 1996	14	46.0
w5354	1995–1996	Female	2	11 Jan. 1996	17	40.0
w5355	1995–1996	Female	1	12 Jan. 1996	18	37.0

[a]Only Argos location classes 0–3 included.

Table 2. Mean error (±SD), measured in terms of distance from a fixed point at 54.002°S, 38.0567°W, of position fixes obtained from Argos in relation to location class provided by Argos.

Location class	Mean distance (m)	Minimum distance (m)	Maximum distance (m)	*n*
3	1 228 ± 957	383	5 368	25
2	1 115 ± 362	467	1 763	21
1	1 566 ± 1 082	534	6 153	29
0	3 779 ± 2 587	1140	8 595	7
A	18 843 ± 42 604	1077	133 017	14
B	22 841 ± 43 926	2532	131 028	8

Table 3. Results of comparisons made between the dive depth distributions of pairs of Antarctic fur seal individuals and grouped by year and sex.

Comparison	*n*	Mean *D*	SD
Male 1992–1993 / male 1992–1993	3	0.179	0.0273
Male 1992–1993 / female 1992–1993	51	0.402	0.1219
Male 1992–1993 / female 1994–1995	21	0.288	0.1016
Male 1992–1993 / female 1995–1996	54	0.466	0.1564
Female 1992–1993 / female 1992–1993	136	0.171	0.0849
Female 1992–1993 / female 1994–1995	119	0.263	0.1196
Female 1992–1993 / female 1995–1996	306	0.203	0.0996
Female 1994–1995 / female 1994–1995	21	0.223	0.0726
Female 1994–1995 / female 1995–1996	126	0.329	0.1253
Female 1995–1996 / female 1995–1996	153	0.230	0.1148

Note: The mean value of the Kolmogorov–Smirnov *D*-statistic is given as a measure of the absolute difference between the dive depth distributions (the larger the value, the greater the difference).

Satellite tracking

Both male and female fur seals were given flipper tags (Dalton Supplies, Henley-on-Thames, U.K.) and tracked at sea using satellite-linked platform transmitter terminals (PTTs) with the Argos satellite system (Argos 1989).

PTTs were deployed on three adult males that were holding breeding territories during December 1992 (Table 1). Males were immobilised chemically using Zoletil (Boyd et al. 1990) and the PTTs (1.5 kg; Sea Mammal Research Unit, Natural Environment Research Council) were attached to the fur on the dorsal side of the neck with epoxy glue (Fedak et al. 1983). These PTTs measured and relayed the maximum depth of the dive made immediately before a transmission. Each transmission received by the satellite contained this information as a digital string in addition to the unique identification code of each transmitter. Depth was measured to an accuracy of 1 m from 10 to 35 m and to 5 m for depths >35 m. The maximum depth resolution was 250 m. Information about dives to <10 m was not transmitted to the satellite because of the low transmission rates available with the Argos satellite system. The transmitter only operated when the antenna was above the water because a submergence sensor near the base of the antenna prevented transmission while it was submerged. When at the surface, a signal was sent every 45 s (no duty cycling), but transmissions stopped 4 h after the seal had left the water.

Information on dive depth was obtained from 17 adult females in 1992–1993 to allow comparison of dive depths with those obtained concurrently from males. Time–depth recorders (Mk III and Mk V, Wildlife Computers, Redmond, Wash., U.S.A.) were programmed to measure depth at 10-s intervals for whole foraging trips. These records were retrieved from the memory (128 kilobytes) of the recorder when seals returned ashore to feed their pups. Purpose-built software

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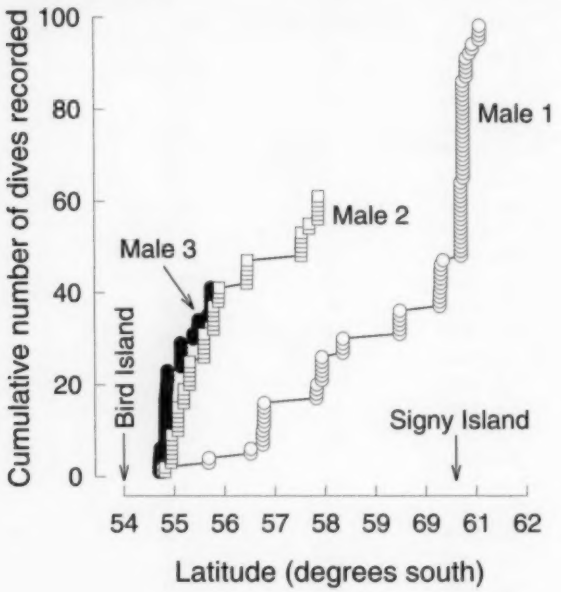
Fig. 2. Cumulative number of dives of three male Antarctic fur seals recorded to >10 m depth that were transmitted to the satellite in relation to latitude.



was also used to analyse this record for individual dives. Dives were defined as excursions below the surface to depths >2 m. However, to provide a comparison with the data obtained from the PTTs on males, only dives to >10 m are presented.

In December–March 1994–1995 and 1995–1996, PTTs (1 W, 250–500 g; Wildlife Computers) were deployed on five and 16 lactating adult females, respectively (Table 1). PTTs were deployed for two to four consecutive foraging trips (5–19 days) while females were foraging to support themselves and a dependent pup ashore at Bird Island. Females were restrained by the method described by Gentry and Holt (1982) and PTTs were attached with epoxy glue in the same way as for adult males except that the instruments were positioned in the middle of the back along the dorsal midline. PTTs on females also incorporated a data logger (256 kilobytes of memory) that recorded depth measured (accuracy 1 m) at 10-s intervals throughout the period of deployment or until the memory was exhausted (10–15 days). These data were recovered from the logger when the instrument was removed at the end of the deployment period. Depth information was used to reconstruct a continuous record of diving during the deployments using purpose-built software (Boyd 1996). As for the PTTs placed on males, those on females had a minimum transmission interval of 45 s (no duty cycling) and they also only operated when the antenna was above the water because of a submergence sensor close to the base of the antenna. The instrument stopped operating after 1 h without submergence.

Accuracy of geographical locations from PTTs and data analysis

The geographical location of animals was calculated from the Doppler shift of each transmission received by the satellite and from the reception of a minimum of three transmissions during each satellite pass. The location of the transmitter was estimated to have a precision of 0.001° of latitude and longitude (111 m). The Argos system divided the precision of calculated locations into six classes representing a gradation from high (class 3) to low confidence (class B) (Table 2). We quantified the degree of error associated with each of these classes by examining the locations received from two PTTs placed at the site of deployment which was fixed using the Global Positioning System.

The average location provided by Argos for the transmitters placed at a known location was within 1–2 km for location classes 1, 2, and 3 (Table 2). The maximum distance from the site for these three location classes was 6 km. On average, location class 0 gave a position 3.8 km from the actual location, with a maximum of 8.6 km (Table 2). Location classes A and B were clearly less precise (Table 2), with maximum potential errors of 130 km. Since we expected animals to be moving over ranges of 100–1000 km, we rejected the location classes A and B as having insufficient reliability. Therefore, the remainder of the analysis used location classes 0–3 only.

To examine the distribution of females when at sea, the position of each dive made by females during 1996 was estimated by interpolation along a direct line between successive locations provided by the satellite. This assumed that animals moved at approximately constant speeds between successive points and that the position of each dive could be obtained from the time of the dive as an indication of the distance travelled between successive position fixes. The position determined in this way was rounded to the nearest 0.1° of latitude or longitude (11 km).

Assumptions and statistics

When comparing the distributions of dive depths among different sexes and years, it is necessary to account for differences among individuals. The lack of normality in the distributions of dive depths meant that the nonparametric Kolmogorov–Smirnov test was used to examine differences between the distributions of dive depths among individuals and groups. Since the Kolmogorov–Smirnov D-statistic is a measure of the absolute difference between two distributions, comparisons among years and between the sexes were made using the mean D-statistic for paired comparisons between individuals in each group. Therefore, by comparing the results of comparisons between the pooled samples for each age and sex class with the comparisons at the level of individuals, it was possible to examine the potential effects among sex and year classes.

We have assumed that fur seals only feed when they dive and that diving is primarily associated with feeding or search for food.

Results

Postbreeding movements of males and females

During December–March, adult female Antarctic fur seals were distributed over the continental shelf of South Georgia and beyond the shelf edge to the northwest of the island whereas males travelled south towards the region of the Antarctic Peninsula (Fig. 1). Females ranged up to 350 km from Bird Island and, of the 261 locations, all were clustered in the same region in both years (Fig. 1).

Of the three male fur seals that were tracked, one (male 1, Fig. 1) reached the South Orkney Islands. This animal was also sighted by personnel from the British Antarctic Survey Base at Signy Island. The same individual also made a foraging trip to the south of the South Orkney Islands where it remained over the continental shelf. It then returned to the South Orkney Islands before moving southwest when the transmissions stopped after 50 days of operation (Table 1). Between 11 and 24 January, male 1 transited from Bird Island to Signy Island, a distance of 886 km, at an average speed of 1.0 $m \cdot s^{-1}$. Evidence from the number of dives recorded that were >10 m depth suggests that male 1 did not feed intensively until he reached the shelf region of the South Orkney Islands (Fig. 2).

Male 2 also travelled towards Signy Island, after departing

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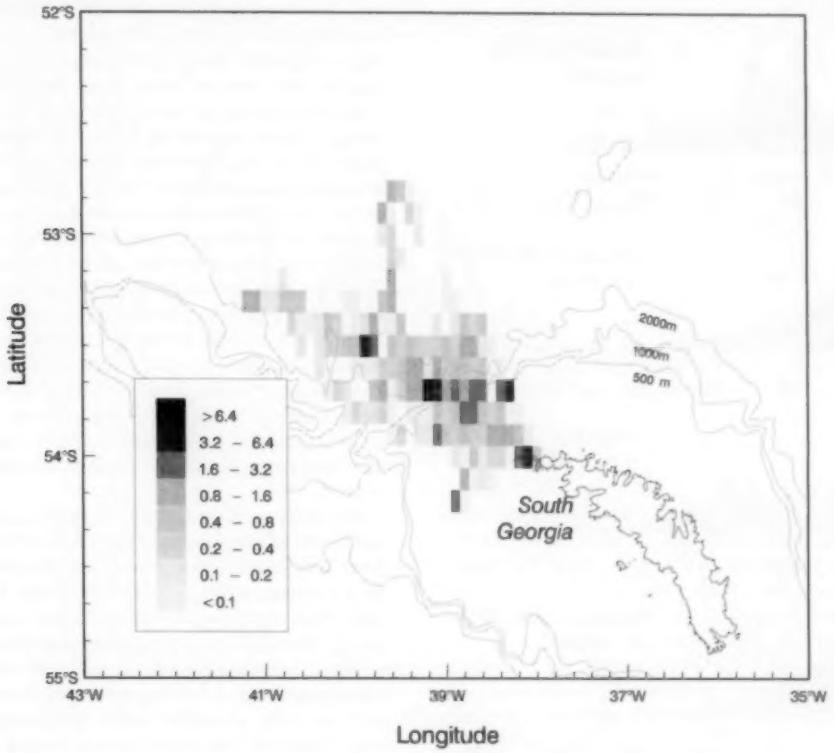
Fig. 3. Interpolated foraging locations of 16 female Antarctic fur seals from Bird Island (54°S, 38°W) during the postbreeding period (December–March) in 1995–1996. The greyscales show the percentage frequency of all dives occurring in each 0.1° × 0.1° square. The 500-, 1000-, and 2000-m isobaths are shown.



South Georgia on 2 January, but transmissions stopped on 22 January when this animal had completed about two thirds of the transit to Signy Island. The mean speed of travel during this period was 0.4 $m \cdot s^{-1}$. Male 3 was tracked between 28 December and 17 January and remained at South Georgia after having initially moved south on a parallel track with the other two seals. He then changed course to the southeast and eventually spent 5 days ashore at a location on the south coast of South Georgia before once again moving southwest approximately parallel with his initial track and with those of males 1 and 2. Soon after leaving South Georgia for a second time, the transmitter on this male stopped. Both males 2 and 3 appeared to feed intensively in the region of South Georgia after leaving Bird Island, as judged by diving activity (Fig. 2).

Male 1 returned to his breeding site at South Georgia during the following winter and male 3 held his territory again during the following two breeding seasons. Male 2 was not seen again. The transmitters from both males 1 and 3 had evidently been lost because the hairs holding the transmitters had broken.

There was a tendency for females to dive more frequently over the shallower zone of the continental shelf to the northwest of South Georgia and over the area of the contental slope, although females also extended their foraging range over deeper water (Fig. 3). However, the dive depths of both males and females were considerably shallower than the ocean depths in the regions in which foraging took place (Fig. 4 compared with Figs. 1 and 3).

Patterns of male abundance

The number of male fur seals ashore at the study site on Bird Island reached a peak in early December and declined through late December and into January (Fig. 5). At Signy Island, there were no male fur seals at the study site through most of November and December, but abundance began to increase through January, with a peak in February. Fur seal abundance declined again to zero by late June (Fig. 5).

Dive depths of fur seals

Although there was overlap in the maximum dive depths of male and female fur seals (Fig. 4), when all dives were combined, the depth distribution of males in 1992–1993 was greater than for females in the same year ($D = 0.468$, $P < 0.002$). This distribution was also different from females in the other two years ($P < 0.001$ in both cases). To examine the effect that variation between individuals may have on these differences, the Kolmogorov–Smirnov D-statistic was also calculated for all possible paired comparisons between individuals and the means were compared among sex and year (Table 3). This showed that 97% (1031/1058) of the comparisons of dive depth distributions were different ($P < 0.05$). The mean D-statistic for paired comparisons between individuals among groups (Table 3) showed that there was an overall greater difference between the dive depth distributions of males in 1992–1993 and females in 1992–1993 and 1995–1996. The dive depth distribution for males in 1992–1993 was

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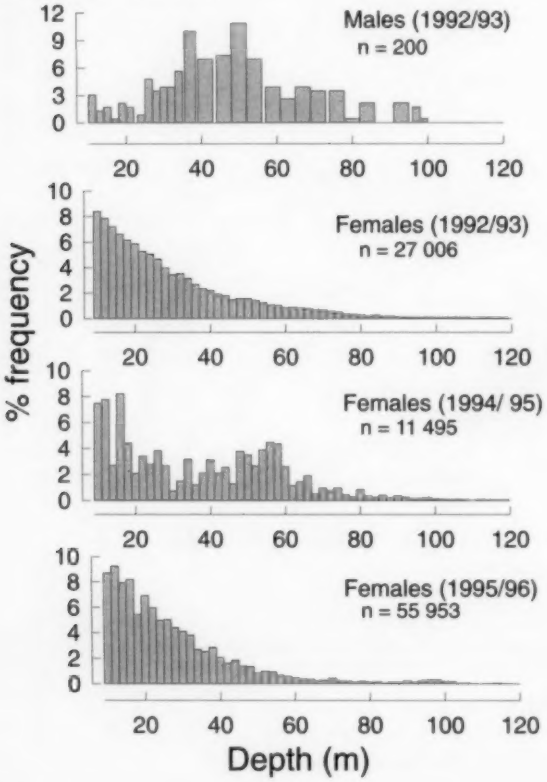
Fig. 4. Frequency distributions of dive depths of the three male Antarctic fur seals tracked during 1992–1993, lactating females during 1992–1993, and the females that were tracked during 1994–1995 and 1995–1996.



more similar to females in 1994–1995. Dive depth distributions for the same sexes within specific years were generally more similar to each other than distributions compared across years and sexes.

Discussion

Geographical distribution

This study has documented the spatial distribution of Antarctic fur seals so that, at least for females, it may be possible to provide a fine-scale (10 km) estimation of their spatial impact on prey resources. It demonstrates that, using intensive deployments of satellite transmitters and appropriate filtering and verification of positional information, the foraging locations of individuals can be measured across consecutive years. This has the potential to add a fine-scale spatial dimension to estimates of predator impact and of potential competition with fisheries.

Although only three males were tracked in one year and, of these, only one animal was observed to haul out at Signy Island, this provides a link between the temporal distribution of numbers of males at South Georgia compared with the South Orkney Islands (Fig. 5). It also seems probable that male 2 eventually moved to the South Orkney Islands and all three males tracked by satellite were located in separate geographical areas from adult females during the postbreeding period of the annual cycle. Both anecdotal observations and counts of animals at South Georgia (Boyd 1989; Reid 1995; this study) suggest that males are either more widely distributed during the postbreeding period or they tend to leave the region of South Georgia at this time. Several lines of evidence suggest that many of these males migrate towards the South Orkney and South Shetland Islands at the end of the breeding season. These include the annual increase in the numbers of males at the South Orkney Islands following the end of the breeding season at South Georgia (Fig. 5), the satellite tracks of the three individuals examined in this study (Fig. 3), and regular observations of male fur seals at the South Orkney Islands with flipper tags that had been applied at South Georgia (British Antarctic Survey archive data). Jablonski et al. (1987) also showed peak numbers of male Antarctic fur seals in the South Shetland Islands during February and March. Moreover, the increasing number of males observed at the South Orkneys since the early 1970s (Smith 1988) has changed in parallel with the increasing numbers at South Georgia where ~96% of the world population breeds (Boyd 1993). This supports the view that the same individual males use both sites at different times of year.

Previous detailed analysis of the foraging behaviour of female Antarctic fur seals (Croxall et al. 1985; Boyd and Croxall 1992; Boyd et al. 1994; Arnould et al. 1996*a*; Boyd 1996) has shown intensive foraging activity during the postbreeding period when most female fur seals are supporting dependent young. Females are restricted in their foraging range by the potential starvation duration of their pups, which remain ashore and have no source of food other than maternal milk until weaning. Therefore, unlike males, females must exploit local resources. This study has shown that males move to forage in different geographical areas during the postbreeding period. Although two of the males tracked in this study appeared to feed close to South Georgia, as judged by their diving activity (Fig. 2), they fed in a different region than females. These males were also apparently feeding in a region where there are no breeding females occupying the adjacent coastline (Boyd 1993).

Differences in dive depths

Programming methods may have biased the estimated dive depth distribution of males. For example, mainly deeper dives will be included if the male fur seals spent more time at the surface between deep dives than between shallow dives because more time will have been available to send information to the satellite after deeper dives. However, since the transmitter was designed to transmit immediately on reaching the surface and dives to <10 m in depth were excluded for both males and females, it seems probable that such a bias was insignificant. Finer-scale data than were available in this study support the view that short and shallow dives are less frequent in males than in females (Boyd and Croxall 1996). Therefore, during 1992–1993, the dive depth distributions of males and females showed that, on average, males dived to greater depths than females and there was, overall, greater similarity between female dive depth distributions among years than there was between the sexes within one of these years.

Based on the larger body size of male fur seals, one would predict that they have increased diving capabilities compared

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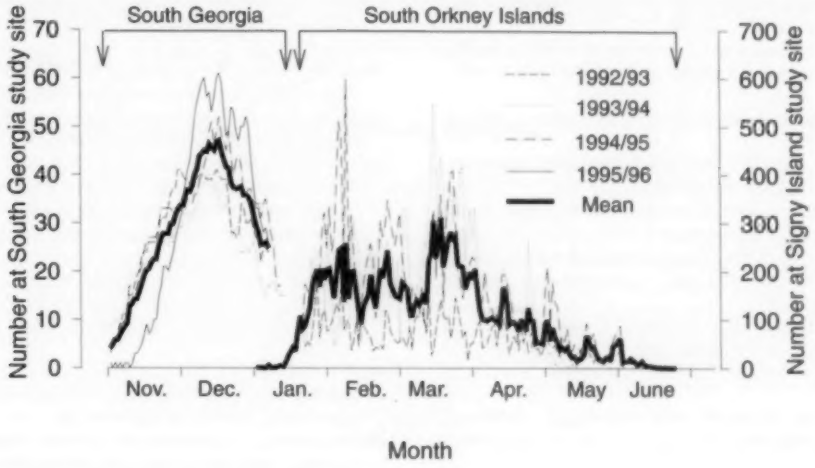
Fig. 5. Number of male Antarctic fur seals counted at two study sites, one on South Georgia on the breeding grounds at Bird Island and another at Signy Island, South Orkney Islands. Thick solid lines show the mean daily counts at each site, and the other lines are the daily counts for each year.



with females (Boyd and Croxall 1996). Therefore, males could exploit the same region of the water column as females, but it is unlikely that the opposite is true. The results of this study and of Boyd and Croxall (1996) suggest that, in general, males do not feed in the same regions of the water column as females. The modal depth of male dives suggests that they are exploiting prey located in the region of the bottom of the surface mixed layer of the ocean (Priddle et al. 1995) whereas females are foraging within the surface mixed layer.

Conclusions

Adult male fur seals have greater absolute energy requirements than females (Costa et al. 1989; Boyd and Duck 1991; Arnould et al. 1996*a*), and this difference influences which prey sources can be exploited profitably by the two sexes. The location of breeding sites is likely to be determined by the local availability of food for lactating females, and such sites are, therefore, likely to be located in close proximity to foraging areas that are optimal for females. The movement of males away from South Georgia during the postbreeding period may reflect either that this is a suboptimal site for males or that foraging by females causes local depletion of resources and that males, which have the option to forage further afield, can forage more successfully in regions where there are no females. It may be significant that there are no important breeding colonies of fur seals at the South Orkney Islands (about six pups born each year at the South Orkney Islands compared with >380 000 at South Georgia; Boyd 1993), even though large breeding colonies of fur seals have developed recently at the nearby South Shetland Islands (Bengtson et al. 1990). This may indicate that the local foraging conditions at the South Orkney Islands are not appropriate for females during nursing or that other local environmental peculiarities of the South Orkney Islands, such as prolonged sea ice cover in some years, may make this site unsuitable for breeding.

Finally, krill fishery data from this region (Everson 1992) show that male and female fur seals from South Georgia are exploiting two of the main regions (South Georgia and South Orkney Islands) where krill fishing takes place. This foraging dispersion is also seen in the black-browed albatross, another summertime krill predator from South Georgia (Prince et al. 1997), and based on the observations of Jablonski et al. (1987), it is also probable that South Georgia fur seals are mainly exploiting the region where krill fisheries occur around the South Shetland Islands. This pattern suggests that both the seals and the fishery are mainly targeting the most abundant exploitable prey.

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Going against the flow: an examination of the propulsive movements made by young brook trout in streams

Robert L. McLaughlin and David L.G. Noakes

Abstract: We examined the propulsive movements and behaviour of young-of-the-year (YOY) brook trout (*Salvelinus fontinalis*) swimming in their natal streams. Our findings demonstrated that swimming performance was influenced by temporal and spatial heterogeneity in water flow. Pectoral fins were used commonly, even by individuals swimming in fast flowing water. There also was spatial variation in the speed attained for a given tail-beat frequency and amplitude. After controlling statistically for variation in spatial location, fork length, and tail-beat amplitude, the swimming speeds brook trout attained for a given tail-beat frequency were lower than values expected from laboratory studies of steady swimming but higher than values expected from laboratory studies of unsteady swimming in standing water. Trout holding station made short-term adjustments in tail-beat frequency also suggesting a degree of unsteady swimming. A field experiment demonstrated that introduction of a current-velocity refuge reduced swimming costs by 10%, on average, without affecting the frequency of foraging attempts made.

Résumé : Chez l'omble de fontaine (*Salvelinus fontalis*), nous avons étudié les mouvements propulsifs et le comportement de jeunes de l'année nageant dans leur cours d'eau natal. Nos résultats ont montré que la performance natatoire était tributaire de l'hétérogénéité temporelle et spatiale de l'écoulement de l'eau. Les nageoires pectorales étaient utilisées fréquemment, même par les individus nageant dans un fort courant. Il y avait aussi une variation spatiale de la vitesse atteinte pour une fréquence et une amplitude données de coups de queue. Après contrôle statistique de la variation de la localisation spatiale, de la longueur à la fourche et de l'amplitude des coups de queue, les vitesses de nage atteintes par les ombles de fontaine pour une fréquence donnée de coups de queue étaient inférieures aux valeurs attendues dans le cadre d'études de laboratoire sur la nage stationnaire, mais supérieures aux valeurs attendues dans le cadre d'études de laboratoire sur la nage instationnaire en eau tranquille. Les ombles nageant sur place faisaient de brefs ajustements de la fréquence de leurs coups de queue, ce qui laisse penser que leur nage était à un certain degré instationnaire. Une expérience sur le terrain a montré que l'introduction d'un abri contre le courant réduisait les coûts de la nage de 10 % en moyenne sans modifier la fréquence des tentatives d'alimentation. [Traduit par la Rédaction]

Introduction

There is an extensive and valuable literature on the locomotion and swimming performance of fishes (Webb 1975; Hoar and Randall 1978; Blake 1983; Webb 1984, 1988). Yet, our understanding of swimming behaviour remains influenced heavily by observations and experiments carried out in controlled, laboratory conditions. Our knowledge of swimming behaviour under more complex natural conditions is not nearly as thorough, in part because the swimming behaviour of most fishes is much more difficult to quantify in the field.

The heterogeneities in physical structure and water flow characterizing natural environments can influence swimming behaviour and performance (e.g., Fausch 1993; Webb 1993*a*). Nevertheless, these effects have not been the focus of much direct attention (but see Webb 1993*a*). Laboratory studies have typically sought to minimize heterogeneities in flow and to focus on steady swimming (e.g., Bainbridge 1958; Brett 1964). Some field studies also have sought to circumvent such effects. Studies of the energetics of stream-dwelling salmonids, for example, have measured tail-beat frequencies of fish in the field and relied on empirical, predictive equations from the laboratory to estimate swimming speed and energetic costs (energy per unit time) from tail-beat frequency (e.g., Feldmeth and Jenkins 1973; Feldmeth 1983; Puckett and Dill 1985). This approach assumes that predictive equations from the laboratory or seminatural stream channels apply to fish swimming in the field, but some fish biologists have cautioned against making such an assumption (e.g., Lindsey 1978; Tang and Boisclair 1993). The importance of such caution is demonstrated by recent comparisons of the energetic costs of forced (steady) swimming in the laboratory and the routine (unsteady) swimming often employed by fish in the field. The latter has been estimated to be 3–14 times greater than the former (Webb 1991; Boisclair and Tang 1993; Krohn and Boisclair 1994).

Field ecologists have recognized that stream fishes exploit boundary layers created by objects in the water column (Fausch 1993, and references therein). These current-velocity refuges (sensu Fausch 1993) are believed to allow fish to hold station in currents of low velocity and capture drift (prey) from adjacent currents of high velocity, thereby improving their net rate of energy gain (Fausch 1993 and references therein).

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Nevertheless, what we know about the significance of velocity refuges has been learned largely by observation (Fausch 1993). In a study of juvenile steelhead (*Oncorhynchus mykiss*) and coho salmon (*O. kisutch*), Fausch demonstrated experimentally that Plexiglas structures placed in the stream reduced current velocity by 49–90%, but it was unclear whether these structures corresponded in size with those the fish would use naturally. Furthermore, earlier studies did not consider how physical structures affect a fish's ability to see drifting prey, even though structures may function to keep a fish out of view from competitors or predators (Fausch 1993).

We used video recordings to examine the swimming behaviour of young-of-the-year (YOY) brook trout (*Salvelinus fontinalis*) in their natal streams. Our study was motivated by the proposition that our understanding of swimming behaviour could be improved by additional exploration of fishes swimming in natural habitats. We sought to accomplish three tasks. First, we used a survey of fish from locations differing in flow and physical conditions to assess how swimming speed related to tail-beat frequency and amplitude, fork length, location in the water column, and substrate type. We then compared these findings with those from studies examining steady and unsteady swimming in the laboratory. Second, we examined the short-term temporal variability in tail-beat frequencies and amplitudes of fish holding station by swimming against the current and of fish moving either upstream or downstream relative to when they held station. Third, we examined experimentally how individuals altered their tail-beat frequency, tail-beat amplitude, and foraging frequency in response to the introduction of a physical structure that would create a current-velocity refuge. A published equation was used to estimate the proportional reduction in energy expenditures accruing from the use of a refuge.

Materials and methods

Field measurements

Data were collected from 23 April to 20 May 1994 and from 3 May to 14 June 1995 at two study sites located on the Credit River drainage system: the West Branch of the Credit River 2 km northeast of Erin, Ont. (43°45′N, 80°07′W), and the North Branch of the Credit River upstream of Highway 24 near Caledon, Ont. (43°52′N, 80°00′W). Studies of the ecology and behaviour of young-of-the-year brook trout have been conducted at these sites since 1984. Fish were observed and handled using protocols approved by the Animal Care Committee, University of Guelph.

YOY brook trout provide an excellent opportunity to examine swimming behaviour under natural conditions. They are easy to observe, because they are not very wary, occur in large numbers in clear shallow water, and tend to hold station by swimming against the water current or by hovering in still water. Swimming speed therefore can be estimated from current velocity. The fish also can be found in locations differing in current velocity and substrate type (Grant and Noakes 1988; McLaughlin and Grant 1994). Finally, there is a significant laboratory literature examining salmonids in stream channels with which to compare our field observations (e.g., Bainbridge 1958; Webb 1971, 1991; Webb et al. 1984).

Our sampling was confined to bright, sunny days; under other weather conditions, glare and poor lighting made it difficult to obtain suitable video recordings of the fish. Each day we tried to select representative samples of fish from different habitats by moving among the broad habitat types available (pool, riffle) and working upstream through each of these, sampling focal individuals. We focused our effort on individual fish that were not moving extensively relative to the stream bottom so that a fish's swimming speed could be approximated by the current velocity at the fish's station. This was particularly relevant to stillwater pools where a fraction of individuals use an active search tactic while foraging and thus spend much of their time moving relative to the stream bottom (McLaughlin et al. 1994). Focal individuals (Martin and Bateson 1993) were selected haphazardly in relation to fish size and the physical conditions at the site. Individual fish were not marked, but we believe the probability of resampling the same fish was small because densities of fish were high, and our sampling was spread out over stream segments 0.5–1 km in length.

Each focal fish was video recorded from a distance of 1.1–2.5 m by an observer standing or kneeling on the bank and operating a Sony SVO-140 8 mm videocassette recorder with a zoom option roughly comparable with a 400-mm lens on a 35-mm camera. We tried to record each fish for at least 30 s from as much above as possible without entering the water and scaring the fish. While video recording the focal fish, we recorded on paper the fish's orientation in the camera viewfinder and its location in the water column. Orientation was quantified as a compass direction (e.g., NNW). The fish's location was quantified as 0.10, 0.25, 0.50, 0.75, or 0.90, the values representing the proportional distance between the fish and the substrate relative to overall water depth. Hence, 0.10 indicated the fish was just above the stream bottom, while 0.90 indicated it was just below the water surface. Immediately after video recording the focal fish, we quantified its fork (body) length, and the current velocity at its station, water depth, and substrate type over which it was swimming. Fork length was estimated visually. To assess the accuracy of our length estimates, approximately 10 individuals were captured each day, using aquarium dip nets, measured for fork length, and then released. In both 1994 and 1995, our estimates of fork lengths (EFL) were good predictors of measured fork lengths (MFL) (r_s = 0.93 and 0.92, df = 35 and 65, respectively). Regression coefficients for EFL versus MFL were not significantly different from 1, and the intercepts not significantly different from 0. Median absolute error was 1.0 mm in both 1994 and 1995. Current velocity at the location of the fish's nose was quantified over 1 min using a Teledyne Pygmy current meter (2 cm deep × 5 cm wide). The meter was calibrated at the Canadian Centre for Inland Waters, Burlington, Ont., prior to each field season. With very slow currents, we assessed current velocity by releasing silt particles into the water column. Current velocity was assigned as 0 $cm \cdot s^{-1}$ if the particles fell straight down and 1 $cm \cdot s^{-1}$ otherwise. Water depth was measured to the nearest centimetre using a metre stick. Substrate type was classified as silt, sand, gravel, or cobble based on size (Gordon et al. 1992, table 5.6), or wood.

In 1995, we experimentally altered the hydrodynamic environment experienced by 23 trout. This entailed video recording the focal fish, measuring the site as above, then placing either a cobblestone (7 occasions) or a stick (16 occasions) extending upward from the substrate 5 cm in front of the location where the focal fish had held station. The cobblestone was 6.5 × 5.8 × 4.1 cm. The stick was 1.3 cm in diameter and approximately 7.5 cm high after being placed in the substrate. These items were typical of the structures young brook trout use at our sites, although many individuals do not use an obvious current-velocity refuge. Once each focal fish returned to the site, we made a second video recording and remeasured the current velocity at its station.

In 1995, additional field measurements also were made to assess the repeatability of our current-velocity measurements. They entailed selecting 100 sites in the field, quantifying current velocity as above, removing and reinserting the current velocity meter, and quantifying the current velocity again. The current meter was also video recorded during the first measurement. Measurement sites were selected haphazardly with respect to current velocity and location in the water column and were representative of the variation young brook trout encounter.

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Table 1. Statistics summarizing the propulsive movements, swimming speeds, body lengths, and local habitats of YOY brook trout sampled in our study.

Variable	Mean	Minimum	Maximum	Repeatability
Tail-beat frequency (beats·s^{-1})	5.53	2.31	8.44	0.75
Tail-beat amplitude (relative to fork length)	0.21	0.09	0.40	0.35
Swimming (current) speed (cm·s^{-1})	5.8	0	21.2	0.99, 0.93[a]
Fork length (cm)	2.8	2.0	4.0	—
Location in the water column	0.34	0.10	0.90	—
Water depth (cm)	12.0	1.0F	40.0	—

[a]The first measurement is for speed averaged over a 1-min period, the second over a 2-s period (see Materials and methods).

Analysis of video

Video recordings were analyzed frame by frame using either an Panasonic or Sony playback machine and either a Panasonic WV-5380 black and white or a Panasonic CT-1330M colour video monitor. The number of tail beats exhibited by a fish was counted for 2-s segments of video during which the fish did not move relative to the stream bottom. Several 2-s segments of video were sampled per fish, and these measurements were converted to beats per second and used to calculate a mean tail-beat frequency for each fish. In 1994, we sampled three or four segments per fish depending on the amount of suitable video. The video contained periods when fish left their position to capture prey or attack conspecifics, and these periods were not used in our analyses of propulsive movements. In 1995, we sampled three 2-s segments per fish. Three measurements per fish were considered reasonable based on the repeatabilities calculated from the previous year. With our field experiment, the difference between the mean tail-beat frequencies measured before and after creation of the current velocity refuge could be small. To increase the accuracy of the means, five 2-s segments were sampled for each fish before and after introduction of the structure.

Relative tail-beat amplitude was measured as a proportion of a fish's length for four tail beats. This entailed finding a frame where the fish's body was straight and measuring its length. We then examined a sequence of frames for a tail beat and measured the distance between the lateralmost excursion of the tip of the tail on one side of the fish's axis of progression and the lateralmost excursion of the tip of the tail on other side. This latter measurement was divided by the former to obtain relative tail amplitude. Our measurements are probably less accurate than those typically obtained in the laboratory, because conditions in the field were more variable and the fish were not recorded from directly overhead. Nevertheless, the mean, minimum, and maximum values we obtained (Table 1) were comparable with those measured in laboratory studies (e.g., Bainbridge 1958; Webb 1975, 1991), and we could control statistically for variation due to differences in the orientation of fish on the screen, if necessary, in our analyses considering how relative amplitude varied with swimming speed.

For a portion of the fish sampled, the video was clear enough that we also could measure the frequency of pectoral fin beats. When possible, these measurements were made over 2-s segments of video.

It was not possible to make all of the measurements for every fish we video recorded owing to variation in factors such as the amount of lighting, the recording distance, whether the fish remained at a station, and its orientation on the screen. Of 329 fish recorded, we measured the frequency of tail beats for 246, relative amplitude for 185, and the frequency of pectoral fin beats for 111. The measurements were made using either the image of the fish or its shadow, whichever was clearest. Durations of video recordings for the fish measured in our field survey were 11–116 s (median 37). Durations of video recordings for fish measured in our field experiment were 31–72 s (median 36) before placement of the stick or cobblestone and 17–72 s (median 45) afterward.

Video recordings of the current meter were used to evaluate current velocity over 2-s intervals in the same way that tail and pectoral fin beats were. The current meter propeller had six cups, and we counted the number of cups moving past a fixed point during three 2-s intervals. These measurements were made because they estimate current velocity on the same time scale as our measurements of tail-beat frequencies, and the values exhibited a similar numerical range to that observed for tail-beat frequencies. The values were then converted to centimetres per second.

Statistical analysis

Several variables were transformed prior to analysis to normalize their distributions as much as possible. Swimming speed, tail-beat frequency, and amplitude were square-root transformed. Fork length was $\log_{10}$ transformed. Descriptive statistics for the fish and the locations where they were observed are provided in Table 1.

Preliminary analyses provided no evidence of significant differences between years so data for 1994 and 1995 were aggregated. The relationships between swimming speed and tail-beat frequency and amplitude, fork length, location in the water column, and the substrate type below the focal individual were examined using analyses of covariance and partial correlations. These analyses were used to examine specific relationships (e.g., swimming speed vs. tail-beat frequency) after controlling statistically for other sources of variation (e.g., fork length) (Sokal and Rohlf 1981). For these analyses, location in the water column and substrate type were treated as nominal (categorical) variables. Including a fish's orientation in these analyses did not have any significant effect and is therefore not included here.

The relationship between swimming speed and tail-beat frequency observed in the field was compared quantitatively with empirical equations published for steady swimming (Hunter and Zweifel 1971; Webb 1971; Webb et al. 1984) and unsteady swimming (Webb 1991) by rainbow trout (*Oncorhynchus mykiss*) in the laboratory. The literature is inconsistent regarding whether swimming (current) speed or tail-beat frequency is considered the dependent variable in analyses of the relationship between tail-beat frequency and swimming speed. We treated swimming speed as the dependent variable because predicting swimming speed from propulsive movements has been more relevant to ecological, field studies (e.g., Feldmeth and Jenkins 1973; Feldmeth 1983; Puckett and Dill 1985). Intraclass correlation coefficients (Kraemer and Korner 1976; Sokal and Rohlf 1981) were used to estimate the repeatabilities of our measurements. Intraclass correlation coefficients represent the correlation expected between any two points selected at random from the same group. The impact of a velocity refuge on energy consumption while swimming was assessed using Puckett and Dill's (1984) equation relating swimming speed (lengths·s^{-1}) to oxygen consumption (O_2·g^{-1}·h^{-1}) for 4-cm coho salmon. This equation was selected because the coho salmon in Puckett and Dill's (1984, 1985) studies were similar in size and ecology to the brook trout we observed. Their equation is for temperatures of 14–15°C and temperatures at the stations of our experimental fish were 11–15°C (mean 13°C). Our analysis was not directed at estimating the energetic costs per se but the relative change in cost due to the use of a velocity refuge.

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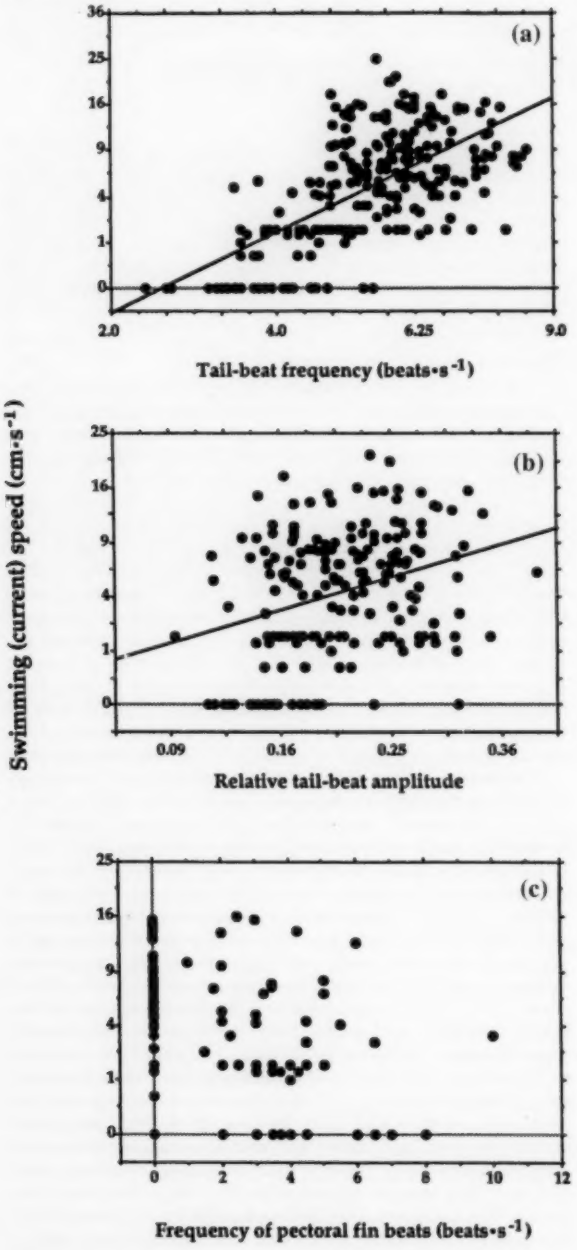
Fig. 1. Relationships between swimming (current) speed and tail-beat frequency (*a*), tail-beat amplitude relative to fork length (*b*), and the frequency of pectoral fin beats (*c*). Each point represents an individual. Swimming speed, tail-beat frequency, and relative tail-beat amplitude are displayed on a square-root scale. For visual purposes, least-square regression lines are provided in Figs. 1*a* and 1*b*.



Results

Preliminary analyses of tail-beat frequency and tail-beat amplitude revealed four noteworthy findings. First, tail-beat frequency (Fig. 1*a*) and amplitude (Fig. 1*b*) were both greater, on average, when trout swam faster than when they swam slower ($r = 0.65$, $P < 0.00001$, df = 242 and $r = 0.31$, $P < 0.0001$, df = 181, respectively). They were also positively, but weakly, correlated with each other (tail-beat frequency vs. amplitude: $r = 0.29$, $P < 0.0001$, df = 180). Second, pectoral fin use declined significantly as swimming speed increased ($r_s = -0.39$, $P < 0.001$, $N = 110$), although individuals using their pectoral fins were observed across the entire range of swimming (current) speeds (Fig. 1*c*). Third, trout hovering in still water nevertheless beat their tails 4.08 times·s^{-1}, on average, and this coincided with active use of their pectoral fins, 4.35 beats·s^{-1} on average. Fourth, there was considerable scatter in the relationships.

Table 2. Partial correlations between swimming speed, tail-beat frequency and amplitude, body size, and measures of where an individual was located ($N = 173$).

Variable	Partial correlation	*F*	*P*
Tail-beat frequency	0.63	107	0.001
Relative tail-beat amplitude	0.17	4.69	0.032
Fork length	0.32	18.2	0.001
Location in the water column	0.17[a]	5.09	0.001
Substrate type	0.28[a]	14.1	0.001
Overall (*R*)	0.82	32.0	0.001

[a]Technically, a partial correlation cannot be calculated for a nominal (categorical) variable; however, we have provided $[F/(F + df_{error})]^{0.5}$ for comparative purposes.

Sixty-eight percent of the variation in estimated swimming speed was accounted for by tail-beat frequency, tail-beat amplitude, fork length, the location where the focal individual was positioned in the water column, and the substrate type over which it swam (Table 2). After controlling statistically for other sources of variation, tail-beat frequency and tail-beat amplitude both remained positively correlated with swimming speed, although the latter relationship was weak (Table 2). For a given level of swimming effort (i.e., tail-beat frequency and amplitude) and set of physical conditions (i.e., substrate type and location), larger trout swam faster than smaller trout (Table 2). Also, for a given level of effort, trout located lower in the water column swam faster than those located higher in the water column (Table 2; least-squares means = 7.5, 4.9, 4.7, 4.7, and 4.8 cm·s^{-1} for the 0.10, 0.25, 0.50, 0.75, and 0.90 locations, respectively). The mean speed for fish near the substrate was significantly greater than for those higher in the water column (linear contrast: $t = 4.05$, df = 162, $P < 0.0001$). Finally, after controlling statistically for swimming effort, location, and fork length, swimming speed varied significantly across substrate types (Table 2; least-squares means = 3.1, 4.6, 6.0, 7.3, and 7.4 cm·s^{-1} for silt, wood, stone, gravel, and sand, respectively).

We compared the swimming speed – tail beat relationship obtained after controlling statistically for variation in tail-beat amplitude, fork length, location in the water column, and substrate type with equivalent relationships reported from the laboratory (Fig. 2). The best fit between the speeds we observed and those predicted based on rainbow trout observed in the laboratory occurred with Webb's (1971) equation (Table 3). It was the only equation for which the regression relating our observed speeds with predicted speeds had a regression

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Fig. 2. Leverage plot of the relationship between swimming (current) speed (SS) and tail-beat frequency (TBF) for YOY brook trout after controlling statistically for variation in a fish's tail-beat amplitude, fork length, location in the water column, and the substrate over which it swam (Table 2). The lines represent predictions of the mean speed expected for a given tail-beat frequency from empirical, laboratory equations developed for steady swimming (Hunter and Zweifel 1971; Webb et al. 1984) and unsteady swimming (Webb 1991). Observed and expected swimming speeds are based on a fork length of 2.8 cm, the geometric mean for the fish we sampled. The regression equation for the line presented for this study is SS = –4.72 + 1.79(TBF).

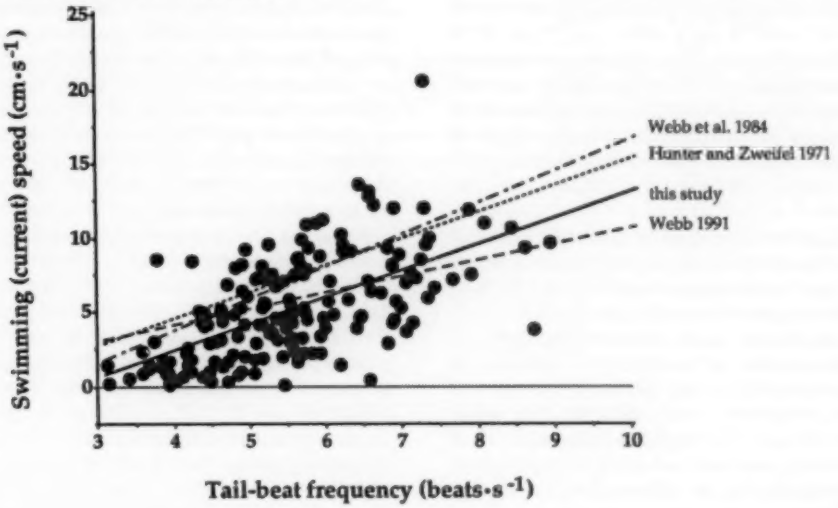

Table 3. Regression parameters and 95% confidence limits for regression equations comparing the swimming speeds of brook trout in Fig. 2 (*Y* value) with values of swimming speed predicted by swimming speed – tail beat relationships developed for rainbow trout in the laboratory (*X* value) (see Fig. 2).

Intercept	Regression coefficient	Range of speeds considered (lengths·s^{-1})	Source
–2.18 (–3.67, –0.68)	1.01 (0.81, 1.20)	2–15	Hunter and Zweifel's (1971) reanalysis of Bainbridge (1958)
–0.38 (–1.54, 0.78)	0.87 (0.70, 1.04)	0–3	Webb (1971)
–0.66 (–1.88, 0.55)	0.83 (0.67, 0.99)	0.2–7.0	Webb et al. (1984)
–4.08 (–5.94, –2.23)	1.64 (1.32, 1.96)	na	Webb (1991)

Note: The range of swimming speeds considered by each source is also provided. Observed and predicted speeds do not differ significantly if the 95% confidence limits for the intercept encompass 0 and the 95% confidence limits for the regression coefficient encompass 1. na, not available

coefficient (slope) not significantly different from 1 and a regression intercept not significantly different from 0 (Table 3). The correlation coefficient was moderate in magnitude (r = 0.61, $P < 0.0001$, df = 174). When compared with values predicted by other equations for steady swimming (Hunter and Zweifel 1971; Webb et al. 1984), the speeds of brook trout at a given tail-beat frequency tended to be significantly lower than expected (Table 3, Fig. 2). When compared with values predicted for unsteady swimming (Webb 1991), the swimming speeds of brook trout tended to be lower than expected at low tail-beat frequencies and higher than expected at high tail-beat frequencies (Table 3, Fig. 2).

There was noteworthy temporal variability in the tail-beat frequencies and amplitudes exhibited by the trout. For 49% of the fish, tail-beat frequencies observed for the same individual varied by 1.25 beats·s^{-1} or more from one 2-s interval to another, and for one fish it was 5.25 beats·s^{-1}. The repeatability of our measurements of tail-beat frequency and amplitude were also noticeably lower than those for current velocity, even when current velocity was measured over 2-s intervals (Table 1). The variation in tail-beat frequency was not due to measurement error. For 50 randomly selected fish, we remeasured tail-beat frequencies during the same 2-s intervals. The variation among intervals for the same fish was significantly greater than our measurement error ($F = 2.58$, $P < 0.0001$, df = 100, 148).

Movement relative to the stream bottom was also powered by changes in tail-beat frequency. Thirteen individuals spent some time moving downstream while facing the current, as well as some time holding station. Their tail-beat frequencies were 0.71 beats·s^{-1} (10%) lower, on average, while moving downstream than while holding station (paired $t = 1.77$, one-tailed $P = 0.05$). Thirty-three individuals spent some time moving upstream as well as holding position. Their tail-beat frequencies were 0.52 beats·s^{-1} (13%) higher, on average, while moving upstream than while holding station (paired t = 1.99, one-tailed $P = 0.028$).

Introducing a physical structure in front of a fish's station reduced current velocity at the station by 1.7 ± 0.63 cm·s^{-1} (mean ± SE). The trout correspondingly reduced their tail-beat

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frequency by 0.51 ± 0.17 beats·s^{-1}, or 8%, (paired t test: t = –2.94, $P < 0.008$, df = 22), however, there was no detectable change in tail-beat amplitude (mean = 0, SE = 0.02, paired $t = 0.19$, $P > 0.80$). The mean reduction in tail-beat frequency for the cobblestone was 0.76 ± 0.33 beats·s^{-1} and for the stick was 0.40 ± 0.20 beats·s^{-1}. The mean reductions did not differ significantly between the two types of structure ($t = 0.96$, $P > 0.35$, df = 21).

Overall, the presence of a physical structure was estimated to reduce oxygen consumption, and therefore energy expenditures, by 13.3 ± 0.04% on average, based on reductions in current velocity, and by 10.3 ± 0.03% on average, based on reductions in tail-beat frequency. Both estimates were significantly greater than zero ($t = 2.78$ and $t = 2.35$, $P < 0.05$, respectively).

Introducing a physical structure had no detectable, negative effect on a fish's ability to capture prey. The number of foraging attempts made per minute ranged from 0 to 11.6 prior to the introduction of a physical structure and from 0 to 10.3 afterward. The mean change in the number of foraging attempts made per minute before versus after the introduction of a physical structure was 0.6 ± 0.7, and this was not significant statistically (paired $t = 0.87$, $P > 0.35$, df = 21). The mean change following introduction of the cobblestone was –0.6 ± 1.2 attempts·min^{-1} and for the stick 1.0 ± 0.8 attempts·min^{-1}. The change in foraging attempts per minute did not differ significantly between the two types of structure ($t = 1.02$, $P > 0.30$, df = 20).

Discussion

Our findings demonstrate that the swimming performance of YOY brook trout in the field is influenced by temporal and spatial heterogeneities in water flow. Our findings also demonstrate that use of current-velocity refuges reduces swimming (energetic) costs moderately while having no obvious effect on the ability to capture drifting prey.

Four lines of evidence support our conclusion regarding the effect of temporal and spatial heterogeneity in water flow. First, a fraction of fish swimming against fast currents beat their pectoral fins frequently. Based on our observations of the video, they appeared to be relying on pectoral fins to maintain a general, upstream heading in seemingly turbulent flow. In the laboratory, however, pectoral fins are apparently not used extensively at tail-beat frequencies above 3 beats·s^{-1} (Hunter and Zweifel 1971). The more extensive use of pectoral fins observed in our study suggests stability, the underlying control problem at slow speeds and in low turbulence (Videler and Wardle 1991; Webb 1993*b*), is an important aspect of swimming in the field.

Second, our correlational analysis revealed spatial variation in the swimming speed attained for a given size and tail-beat frequency and amplitude. We believe the differences related to a fish's location in the water column reflect the ability of these small fish (0.5 cm deep × 0.4 cm wide) to exploit boundary layers above the substrate, which we could not measure accurately with our larger current meter (2 cm deep × 5 cm wide). At a specific site, water speed and hence tail-beat frequency would be expected to be lower, on average, near the stream bottom than away from it. Therefore, if we measured accurately the swimming (current) speed at the fish's location, no relationship between speed and location in the water column would be expected after controlling for tail-beat frequency. If, however, we overestimated the swimming (current) speed of fish located just above the stream bottom, then fish located one tenth of the distance from the stream bottom to the surface would appear to attain higher swimming speeds for a given tail-beat frequency, just as we observed. Accounting for the variation across substrate types is more challenging. It may reflect differences in the prevalence or size of suitable boundary layers among microhabitats differing in substrate type. Alternatively, it may reflect the effects rearing environment (e.g., substrate type, hydrodynamic environment) can have on swimming performance (Bams 1967).

Third, our quantitative analysis of the swimming speed – tail beat relationship suggests YOY brook trout in the field exhibit some degree of unsteady swimming, because the speed attained for a particular tail-beat frequency tended to be lower than that expected for steady swimming in laboratory stream channels but generally higher than that expected for unsteady swimming in standing water. This is an interesting finding because holding station against a stream current is a commonly encountered example of where steady swimming is expected to predominate (e.g., Webb 1991). On one hand, such comparisons should be interpreted cautiously because the field and laboratory environments differ in a variety of ways. On the other hand, it is noteworthy that the qualitative relationships observed between tail-beat frequency and amplitude, fork length, and swimming speed are consistent with those reported in laboratory studies (e.g., Bainbridge 1958; Stevens 1979; Webb 1975; Webb et al. 1984). As well, the quantitative relationship we obtained for brook trout in the field (Fig. 2) is reasonably close to those obtained for rainbow trout in the laboratory.

Fourth, YOY brook trout exhibited noteworthy, temporal variability in tail-beat frequency and amplitude. In fact, the short-term differences in tail-beat frequency exhibited by trout holding station were similar in magnitude to the adjustments made in mean tail-beat frequency by trout moving upstream or downstream to a new station. Based on our experience observing these fish (this study; McLaughlin and Grant 1994), we believe the variation reflects adjustments in swimming effort in response to fluctuations in current velocity. Ebbs and surges in current velocity are common in natural streams (Vogel 1981; Gordon et al. 1992). Such short-term fluctuations in current velocity would not be detected with our usual current velocity measurements which average velocity over 60 s. They might also go undetected in our 2-s measurements because the propeller's inertia makes it insensitive to short-term fluctuations in water velocity. Unfortunately, it is presently unclear how the levels of temporal variability we observed compare with the levels exhibited by fish swimming steadily in laboratory stream channels or those swimming in tanks of standing water.

The differences between the propulsive movements observed under field conditions relative to laboratory conditions will likely add significantly to the energetic cost of swimming. Authors of recent studies examining fish swimming unsteadily in standing water have concluded that acceleration and deceleration add considerably to the cost of swimming (Webb 1991; Boisclair and Tang 1993; Krohn and Boisclair 1994). This will probably be true for swimming against an unsteady current as

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well. In addition, the use of pectoral fins to maintain stability will increase drag and decrease the distance covered per tail beat (Videler and Wardle 1991; Webb 1993*b*). Consequently, fish in the field may be expected to work harder than fish in the laboratory to maintain a given average speed, as suggested by Fig. 2. In fact, for those brook trout where we could assess pectoral fin use, fish using their pectoral fins swam significantly slower for a given tail-beat frequency than those not using their pectoral fins (least-square means: 3.9 vs. 5.9 $cm \cdot s^{-1}$, respectively; $F = 7.21$, $P < 0.001$, df = 1, 75).

The findings from our field experiment provide evidence that, by using a current-velocity refuge, YOY brook trout can reduce the cost of holding station moderately without any appreciable effect on their ability to detect drifting prey. This supports the widely accepted idea that velocity refuges allow fish to hold station in currents of low velocity and capture drift (prey) from adjacent currents of high velocity, thereby improving the fish's net rate of energy gain. There are few direct measurements of how velocity refuges affect swimming costs and none of how they affect foraging rates. Fausch (1993) reported that his Plexiglas structures reduced current velocity by 49–90%, compared with 10–13% for YOY brook trout, but Fausch's structures were much larger than the natural barriers used by YOY brook trout. Authors of several other investigations, including one on our study system, found that swimming speeds or tail-beat frequencies are 30–90% lower at locations where fish held stations relative to arbitrary locations nearby (Fausch and White 1981), or locations where the fish captured prey (Bachman 1984; Grant and Noakes 1988). The smaller reduction observed in our study relative to others may be because we quantified the difference in effort exhibited at a location when a structure was present versus when it was not, rather than differences between where food is captured and where a fish holds station.

The 10–13% reduction in energetic costs should be considered an initial approximation because of uncertainties surrounding the prediction of energetic expenditures from swimming speeds, including the fact that Puckett and Dill's (1984) equation was developed under laboratory and not field conditions. Our estimates may be satisfactory providing the cost of swimming in the field is a fixed multiple of the cost of swimming in the laboratory. While this appears to be true for forced swimming versus spontaneous swimming in standing water (Webb 1991; Boisclair and Tang 1993; Krohn and Boisclair 1994), it is unclear whether it is true for unsteady swimming in running water.

Stronger exploration of how fish alter their swimming behaviour in response to physical structure and heterogeneity in water flow could be valuable to at least three areas of research. One is the construction of models examining population dynamics, particularly those that predict the numbers and sizes of individuals reaching a given age by linking subroutines modeling spatial heterogeneity in water flow with subroutines modeling the energetic costs and benefits of using a particular foraging station (e.g., Jager et al. 1993; Van Winkle et al. 1996). A second is habitat management (Fausch 1993). In streams and rivers occupied by salmonids, addition of structures serving as current-velocity refuges increases the number of locations that are energetically suitable for salmonid fishes (Fausch 1993; Shuler et al. 1994), as well as the density and standing crop of the population (Fausch and Northcote 1992; Shuler et al. 1994). A third is the design of fish passageways (Clay 1995). The ability of fishes to use low-velocity areas or structure within the fishway could be important in determining the species and sizes of fishes that pass successfully (e.g., Chase 1996).

Our study represents a demonstrable example of what could be done in the field. The findings of our field survey are currently limited to a small range of sizes, but they are probably relevant to early life stages of other salmonid species in streams. Whether they apply to older life stages is less clear, particularly for species that avoid swimming by seeking shelter behind structures. The findings of our field experiment are also limited to a small number and type of structures that are used as velocity refuges. Moreover, the reduction in current speed and hence tail-beat frequency behind a structure may differ with the current velocity of the main stream channel and the size and life stage of fish examined.

There is a tradition of examining the swimming behaviour of fishes using carefully conducted laboratory experiments. Our study supports the proposition that our understanding of fish locomotion could be improved by going against the flow and exploring the behaviour of fishes swimming in their natural habitat. It questions the perception that trout holding station in the field are swimming steadily against the current. It also demonstrates that, in some circumstances, the effects temporal and spatial heterogeneity in water flow have on swimming performance can be examined more directly in the field.

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Particle retention by non-suspension-feeding cyprinid fishes

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Abstract: Insectivorous Sacramento squawfish (*Ptychocheilus grandis*) and omnivorous benthic-feeding California roach (*Hesperoleucus symmetricus*) were exposed to suspended styrene microspheres (31–90 μm) or brine shrimp cysts (210–300 μm) in the presence of finely crushed Tetramin flakes or adult *Artemia*. These fish species retained small numbers of microspheres, and significantly more brine shrimp cysts than microspheres. During a 10-min period, they swallowed all of the brine shrimp cysts from a volume of water equivalent to 1–15 times their body volume. Squawfish and roach do not possess the morphological features of the branchial apparatus and palate that are associated with suspension feeding in confamilial Sacramento blackfish (*Orthodon microlepidotus*). The brine shrimp cysts could have been trapped between squawfish and roach gill rakers, while the microspheres as well as the brine shrimp cysts could have been retained on mucus-covered buccopharyngeal surfaces. These results suggest that non-suspension-feeding fish species may ingest small suspended particles routinely, with energetic and ecotoxicological implications that deserve further study.

Résumé : Nous avons exposé des spécimens de *Ptychocheilus grandis*, un poisson insectivore, et de *Hesperoleucus symmetricus*, un poisson omnivore se nourrissant sur le fond, à des microsphères de styrène (31–90 μm) en suspension ou à des kystes d'*Artemia* (210–300 μm) en présence de flocons de Tetramin finement broyés ou d'*Artemia* adultes. Les microsphères ont été retenues en petit nombre chez les deux poissons; les kystes d'*Artemia* retenus étaient beaucoup plus nombreux. Au cours d'une période de 10 min, les poissons ont ingéré tous les kystes d'*Artemia* présents dans un volume d'eau équivalant à 1–15 fois leur volume corporel. Ni l'un ni l'autre des deux poissons ne possède les caractéristiques morphologiques propres à l'appareil branchial et au palais d'*Orthodon microlepidotus*, espèce de la même famille qui se nourrit d'organismes en suspension. Il se peut que les kystes aient été coincés entre les branchicténies des sujets, alors que les microsphères, de même que des kystes, pourraient avoir été retenus sur les surfaces buccopharyngiennes couvertes de mucus. D'après ces résultats, il se pourrait que les poissons non suspensivores ingèrent régulièrement des petites particules en suspension, un phénomène dont les conséquences, sur le plan énergétique et écotoxicologique, devraient être étudiées. [Traduit par la Rédaction]

Introduction

The ability of suspension-feeding fishes to filter particles as small as ~10 μm from the water is well established (reviews in Lazzaro 1987 and Sanderson and Wassersug 1993). Despite the extensive data available on suspension-feeding fishes, there have been no quantitative analyses of small particle retention in non-suspension-feeding fishes.

Particle retention by non-suspension-feeding fishes is of interest for a number of reasons: (*i*) the incidental retention of organic particles could serve as a nutritional source, (*ii*) ingestion of and direct contact with inorganic and organic particles are probable uptake routes for xenobiotic chemicals adsorbed to the particles (review in Knezovich et al. 1987; Kolok et al. 1996), and (*iii*) if non-suspension-feeding fishes retain small particles, identification of the structures and behaviors involved in such retention might elucidate the retention mechanisms used by suspension feeders.

Research on particle retention has usually involved placing suspension-feeding fishes in aquaria with known particle concentrations and quantifying the subsequent decline in concentration with time (e.g., Johnson and Vinyard 1987). This experimental protocol has not been used with non-suspension-feeding fishes, since such species have been expected to have little if any effect on particle concentration. However, a different procedure (Sanderson and Cech 1995) can be applied to quantify particle retention in non-suspension-feeding fishes. Fish that have been feeding in an aquarium in the presence of indigestible particles are transferred to separate beakers that lack particles, and the feces of individual fish are then collected by filtration of the water in the beakers. By counting the numbers of particles in the feces, the investigator can quantify the retention of small numbers of particles, which cannot be detected by monitoring particle concentration in the aquarium. In addition, variation in particle retention between individuals can be assessed.

Mucus has been implicated in the retention of particles by suspension-feeding fish species in the families Cichlidae (e.g., Drenner et al. 1987; Sanderson et al. 1996), Cyprinidae (e.g., Sanderson et al. 1991; Hoogenboezem and van den Boogaart 1993), Clupeidae (e.g., White and Bruton 1983; Friedland 1985), Mugilidae (e.g., Harrison and Howes 1991), and Osteoglossidae (e.g., Bauchot et al. 1993). Since the buccopharyngeal cavity of fishes is lined with a mucus membrane

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(Walker et al. 1981; Sibbing and Uribe 1985), non-suspension-feeding fishes may retain particles incidentally on sticky buccopharyngeal surfaces, despite their lack of suspension-feeding behaviors.

Here, we report the particle retention abilities of two cyprinid species that are not suspension feeders, the insectivorous and piscivorous Sacramento squawfish (*Ptychocheilus grandis*) and the benthic-feeding California roach (*Hesperoleucus symmetricus*). For comparison, we conducted experiments on a suspension-feeding cyprinid, Sacramento blackfish (*Orthodon microlepidotus*). The branchial and palatal morphology of these three species was then compared using electron microscopy. *Ptychocheilus*, *Hesperoleucus*, and *Orthodon* have been placed with six other genera and one species in an unresolved polychotomy at the base of the "western clade" of North American cyprinids (Coburn and Cavender 1992).

We tested the following null hypotheses: (*i*) the non-suspension-feeding species, squawfish and roach, do not retain small particles and are therefore not selective in the sizes of small particles retained, (*ii*) there are no significant differences among squawfish individuals or among roach individuals in particle retention, and (*iii*) squawfish, roach, and blackfish have similar branchial and palatal morphologies. The particle retention data and morphological data presented here have not been reported previously, with the exception of the number of particles retained during the consumption of adult *Artemia* by blackfish (Sanderson and Cech 1995).

Materials and methods

Squawfish and roach were seined from Bear Creek, Colusa County, California. Blackfish were seined from a holding pond in central California in which several blackfish had spawned. The experimental protocol of Sanderson and Cech (1995) was followed.

In the first set of experiments, a measured volume of stock solution containing styrene microspheres (Bio-Rad Laboratories Bio-Beads S-X3, 30–90 μm; Drenner et al. 1987) was added to three aquaria (50 × 25 × 30 cm high). Each aquarium contained eight marked fish belonging to one of the three species. The microsphere concentration in the aquaria at the beginning of the experiments was ~25·mL^{-1}. Immediately after the addition of microspheres to the aquaria, 100 mL of water containing 150 dead adult brine shrimp as prey (*Artemia*, 4 mm long) was added to each aquarium. The experiments were repeated with the same fish specimens ~2 weeks later using finely crushed Tetramin flakes (0.1–1.0 mm in diameter) in place of adult *Artemia*. The concentration of crushed Tetramin in the aquaria was 0.1 g·L^{-1}.

In the second set of experiments, *Artemia* cysts that had been hydrated for 2 h were added in place of microspheres to two aquaria containing either the eight marked squawfish or the eight marked roach that were used above. The goal of this second set of experiments was to determine whether particle type influences retention by non-suspension-feeding fish. The cysts were 255 ± 15 μm in diameter (mean ± SD, range 210–300 μm, $N = 200$). The concentration of cysts in the aquaria at the beginning of the experiments was ~23·mL^{-1} for the squawfish and 12·mL^{-1} for the roach. Immediately after the addition of cysts to the aquaria, 100 mL of water containing 150 dead adult *Artemia* (4 mm long) was added to each aquarium. Prior to the addition of *Artemia* to the aquaria, all *Artemia* brood sacs containing eggs were removed with fine dissecting scissors under a dissecting microscope.

Water samples scooped gently into a petri dish from the aquaria during the experiments were examined under light and dissecting microscopes. Microspheres and cysts were neither clumped together nor attached to the *Artemia* or Tetramin.

Ten minutes after the addition of the microspheres or cysts, the submersible pump was turned off and all fish were placed in a bucket of fresh water. Each fish was rinsed externally and placed in a separate beaker containing 1 L of water. Within a few hours, each fish was fed adult *Artemia* from which the brood sacs had been removed.

After 12 h, each fish was rinsed over the beaker and transferred to a separate beaker of fresh water. The water from each of the beakers in which the individual fish had been held was then filtered on a separate filter paper (Nalgene 205-4045, 0.45-μm pore size), and each beaker was rinsed three times over the filter paper. This entire procedure was repeated once or twice, at intervals of 12 h, until the feces were devoid of microspheres or cysts. All of the fish remained healthy.

In preliminary experiments, the water in the beakers was filtered 2–5 h after the fish had been placed in the beakers, before they had defecated. Examination of the filter papers revealed no microspheres or cysts that had been transferred into the beakers from the external surfaces of the fish.

The filter papers were placed under coverslips on slides and moistened with water. A light microscope (100×) with an ocular micrometer was used to measure microspheres from the first set of experiments in 10-μm classes from 31 to 90 μm. All of the microspheres from the feces that were collected during the first set of experiments were sized, with the exception of the microspheres from the crushed Tetramin experiment on blackfish, which were counted but not sized. For the second set of experiments, the ingested cysts were counted but not sized.

G-tests for goodness of fit with Williams' correction were performed, comparing the observed size-class frequency distribution of microspheres in the feces of each species with the frequencies expected if particle encounter and retention were nonselective. The observed frequency distribution for each species was obtained by summing the number of microspheres in each size-class from the feces collected during the first set of experiments. The total number of microspheres sized was 78 for squawfish, 187 for roach, and 4771 for blackfish. The expected frequency distribution was obtained by using a laser particle counter (Granulometre 715) on the solution of microspheres added to the aquaria at the beginning of the experiments.

To confirm that the ingestion of microspheres was not the result of microsphere adherence to adult *Artemia* in the aquaria, the number of *Artemia* swallowed by each individual blackfish during one experiment was compared with the number of microspheres counted in the feces of that individual. A Panasonic WV-2170 video camera was used to videotape (30 frames·s^{-1}) the aquarium during the experiment in which the blackfish were fed adult *Artemia* in the presence of microspheres. A Panasonic AG-1950 VHS player with jog/shuttle was used to analyze the videotape frame by frame to determine the number of *Artemia* swallowed by each individual blackfish.

At the completion of the experiments, all specimens were euthanized with MS 222 and body length and mass measurements were made. Casts of the oral cavities of three specimens of each species were made by injection of silicone sealant. Dry cast volumes were calculated using the masses of known volumes of dry silicone.

The individuals from which oral casts were not taken were preserved in 10% buffered formalin and transferred to a graded ethanol series. Specimens were critical point dried using a Samdri-PVT-3B and coated with 20 nm of gold–palladium in a Hummer VII sputter coater for scanning electron microscopy on an AMRAY 1810.

Results

Behavioral response to prey

The squawfish (4.8–7.4 cm standard length (SL), 1.8–4.9 g),

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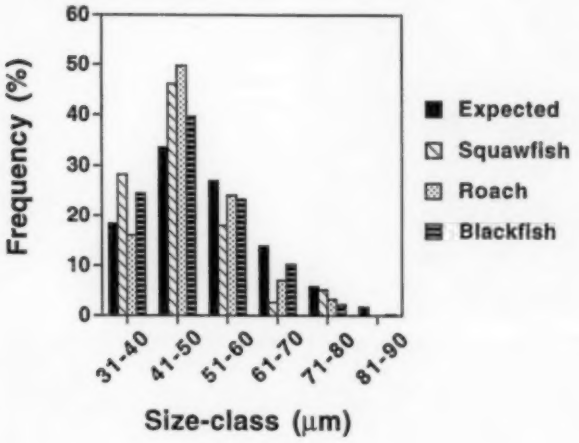
Fig. 1. Observed and expected size-class frequencies of microspheres. The size–frequency distribution of microspheres in the solution that was added to the aquarium at the beginning of each experiment ("expected") was significantly different from the size–frequency distribution in the feces of the Sacramento squawfish and of the California roach but not of the Sacramento blackfish (*G*-tests for goodness of fit: squawfish, $P < 0.001$; roach, $P < 0.01$; blackfish, $P > 0.05$).



roach (4.7–5.8 cm SL, 1.9–3.8 g), and blackfish (4.5–6.6 cm SL, 1.5–4.6 g) consumed the adult *Artemia* using particulate feeding directed at individual prey. None were observed to suspension feed in the presence of adult *Artemia*.

The squawfish and roach continued to respire normally but did not suspension feed or particulate feed in the presence of the crushed Tetramin. Neither the squawfish nor the roach showed the water transport behaviors (ram feeding or repetitive suction) that are always associated with suspension feeding (Lazzaro 1987). However, when crushed Tetramin was added to the aquarium, the blackfish suspension fed by generating suction at a higher frequency and amplitude than occurred during respiration. The blackfish opened and closed their mouths at a frequency of ~3 Hz for several seconds and then respired normally for 1–5 s before resuming feeding.

Microsphere retention

Despite their lack of suspension feeding, squawfish and roach retained small numbers of microspheres in the presence of crushed Tetramin and *Artemia* (Table 1). Blackfish retained more microspheres than did squawfish or roach (two-way ANOVA: $P < 0.0001$). There were no significant differences in particle retention between prey types ($P > 0.05$), nor was there a significant interaction of species with prey type ($P > 0.05$).

Size–frequency distribution of microspheres

There was a significant difference between the size–frequency distribution of microspheres in the solution that was added to the aquarium at the beginning of the experiment and the size–frequency distribution in the feces of the squawfish and of the roach, but not of the blackfish (*G*-tests for goodness of fit: squawfish, $P < 0.001$; roach, $P < 0.01$; blackfish, $P > 0.05$). Both squawfish and roach retained more microspheres than expected in the size range from 41 to 50 μm and fewer microspheres from 51 to 90 μm than expected (Fig. 1). In addition, there was a higher frequency of microspheres from 31 to 40 μm in the feces of squawfish than in the solution that was added to the aquarium.

Table 1. Average number of microspheres retained per individual (±SE, $N = 8$) in the first set of experiments (concentration of microspheres used was ~25·mL^{-1}).

Prey type	Sacramento squawfish	California roach	Sacramento blackfish
Crushed Tetramin	3±1	6±2	912±264
Adult *Artemia*	6±2	17±5	596±110

Table 2. Average number of brine shrimp cysts retained per individual (±SE, $N = 8$) in the second set of experiments, and number of cysts·mL^{-1} (±SE, $N = 3$) in the aquarium during the experiments (prey type was adult *Artemia*).

	No. cysts·individual^{-1}	No. cysts·mL^{-1}
Sacramento squawfish	114±71	23±1
California roach	357±117	12±1

Table 3. Two-way repeated measures ANOVA on data from the first and second sets of experiments on Sacramento squawfish and California roach.

Source	SS	df	*F*	P
Species	166 500	1	3.3069	0.09
Individual	704 891	14	1.0189	0.49
Particle type	549 340	1	11.1163	0.005
Species × particle type	148 995	1	3.0150	0.10
Individual × particle type	691 845	14	606.8147	<0.0001

Brine shrimp cyst retention

Squawfish and roach retained more brine shrimp cysts than microspheres (two-way repeated measures ANOVA: $P = 0.005$, Tables 2 and 3), despite the higher concentration of microspheres than cysts in the aquaria during the experiments. There were no significant differences in particle retention between squawfish and roach or among individuals ($P > 0.05$, Table 3); however, the interaction of individual with particle type was highly significant. For example, a squawfish individual and a roach individual that retained among the fewest microspheres (one to six microspheres during each experiment) retained the most brine shrimp cysts of any individuals of their species (593 and 985 brine shrimp cysts, respectively).

Variation among individuals in particle retention

There were no significant differences among squawfish individuals or among roach individuals in the number of microspheres retained (one-way repeated measures ANOVAs: $P > 0.05$). However, there was high variability in the number of microspheres and brine shrimp cysts retained. The number of microspheres retained by each individual squawfish or roach during a single 10-min experiment varied from 0 to 50, and the number of brine shrimp cysts retained varied from 0 to 985. The number of microspheres or brine shrimp cysts retained by each individual squawfish, roach, and blackfish was not

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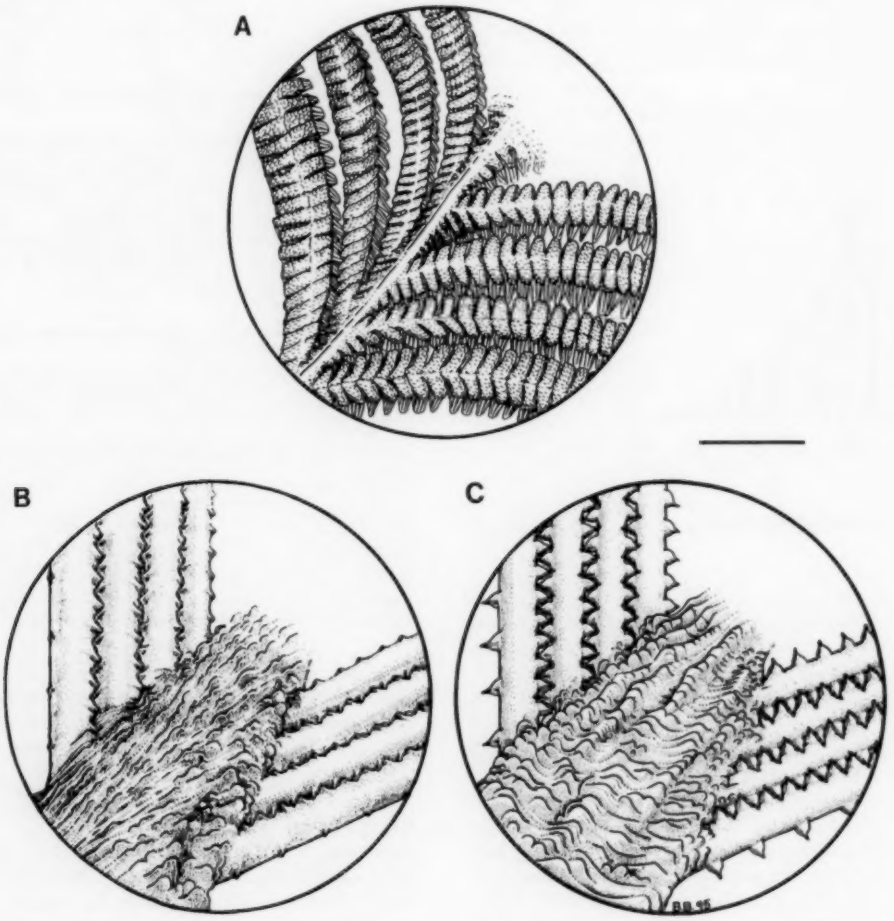
Fig. 2. Dorsal view of the anterior four branchial arches and the floor of the pharynx. Anterior is at the bottom left. Scale bar = 1 mm. (A) The arches from the left and right sides of Sacramento blackfish extend almost to the midline of the pharynx; (B) the pharyngeal floor of Sacramento squawfish is composed of a wide strip of tissue; (C) the pharyngeal floor of California roach is similar to that of Sacramento squawfish (Fig. 2B).



correlated with the standard length or mass of that individual (Pearson product-moment correlations: df = 6, $P > 0.05$). In addition, there was no relationship between the number of microspheres retained by each individual blackfish and the number of *Artemia* swallowed by that individual (least-squares linear regression: $R^2 = 0.01$, $P > 0.05$).

Branchial and palatal morphology

Figure 2 illustrates the anterior four branchial arches. The fifth pair of arches (not shown) forms the pharyngeal jaws. The branchial apparatus of blackfish is distinct from that of squawfish and roach. The strip of tissue along the midline of the pharynx, where the paired hypobranchials of the arches meet the unpaired basibranchials, is very narrow in blackfish (Fig. 2A). The arches from the left and right sides of blackfish extend almost to the midline of the pharynx. In addition, the proximal rakers located at the bases of the second, third, and fourth arches extend along the midline of the pharynx.

The branchial apparatus of squawfish (Fig. 2B) is very similar to that of roach (Fig. 2C). The floor of the pharynx is composed of a wide strip of tissue that supports the basibranchials. Consequently, the branchial arches terminate at a distance lateral to the midline of the pharynx. The proximal rakers of squawfish and roach do not continue onto the basibranchial region of the pharynx.

The gill rakers of blackfish are unusual in that they consist of closely spaced, broomlike tufts that extend along all lateral and medial sides of the four anterior arches (Fig. 3A). In contrast, the gill rakers of squawfish (Fig. 3B) and roach (Fig. 3C) are short, widely spaced protuberances. Roach gill rakers are more pointed than the knobby rakers of squawfish. In both squawfish and roach, the lateral side of the first branchial arch has reduced gill rakers that are spaced about twice as widely as the rakers on the other arches (Figs. 2B and 2C).

The palatal organ of cyprinids is a muscular sheet of tissue that forms the pharyngeal roof, located dorsal to the ends of the branchial arches that are illustrated in Fig. 2. In blackfish, the palatal organ is highly rugose, with many protruding

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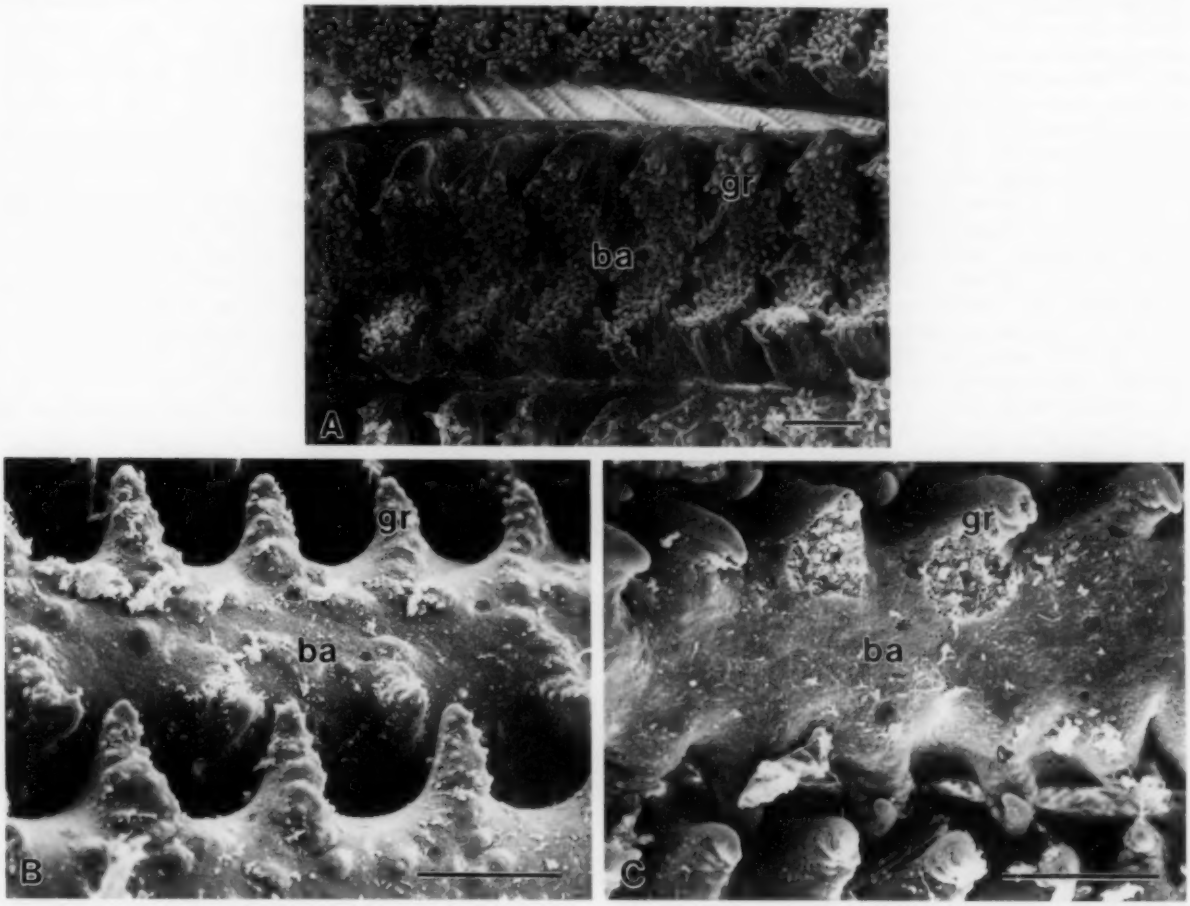
Fig. 3. Scanning electron micrographs of gill rakers (gr) along branchial arches (ba). Scale bars = 200 μm. (A) Tufted, closely spaced gill rakers of Sacramento blackfish; (B) short, widely spaced gill rakers of Sacramento squawfish; (C) short, widely spaced gill rakers of California roach.



papillae (Fig. 4A). The branchial arches of blackfish fit into shallow grooves that are located along the lateral sides of the palatal organ (e.g., top of Fig. 4A).

In squawfish (Fig. 4B) and roach (Fig. 4C), the palatal organ possesses folds that are oriented from anterior to posterior. The tissue is relatively smooth and lacking in papillae. There are no grooves for the branchial arches.

The maximal volume of the oral cavity, as determined from silicone casts, was greater for squawfish (0.20 ± 0.03 mL, 5.2 ± 0.1 cm SL, mean ± SD, $N = 3$) than for roach (0.13 ± 0.01 mL, 5.3 ± 0.2 cm SL) and blackfish (0.14 ± 0.02 mL, 5.5 ± 0.4 cm SL) (one-way ANOVA: $P < 0.02$).

Discussion

Diets

Suspension-feeding fishes have been defined as those species that consume planktonic prey that are too small to be sensed, pursued, and engulfed as individual particles (Lazzaro 1987; Sanderson and Wassersug 1993). Suspension-feeding fishes are identified by the ram-feeding and (or) repetitive suction behaviors used to transport large volumes of water into their mouths or by the presence of minute prey in their stomachs (e.g., phytoplankton or bacteria).

According to the above criteria, neither squawfish nor roach is a suspension feeder. Squawfish in the size range used in this study consume primarily aquatic insects, and occasionally fish (Brown 1990). Roach in the size range used are primarily omnivorous benthic feeders that consume filamentous algae, diatoms, desmids, detritus, aquatic insects, and small crustaceans (Moyle 1976). Blackfish in the size range used are capable of suspension feeding on phytoplankton and zooplankton, benthic feeding on diatoms and crustaceans, and particulate feeding on zooplankton (Moyle 1976).

Particle retention by non-suspension-feeding fishes

On average, squawfish retained 3–6 microspheres·individual^{-1} and roach retained 6–17 microspheres·individual^{-1} (Table 1) during 10 min of exposure to water containing 25 microspheres·mL^{-1}. Under the same conditions, blackfish retained about two

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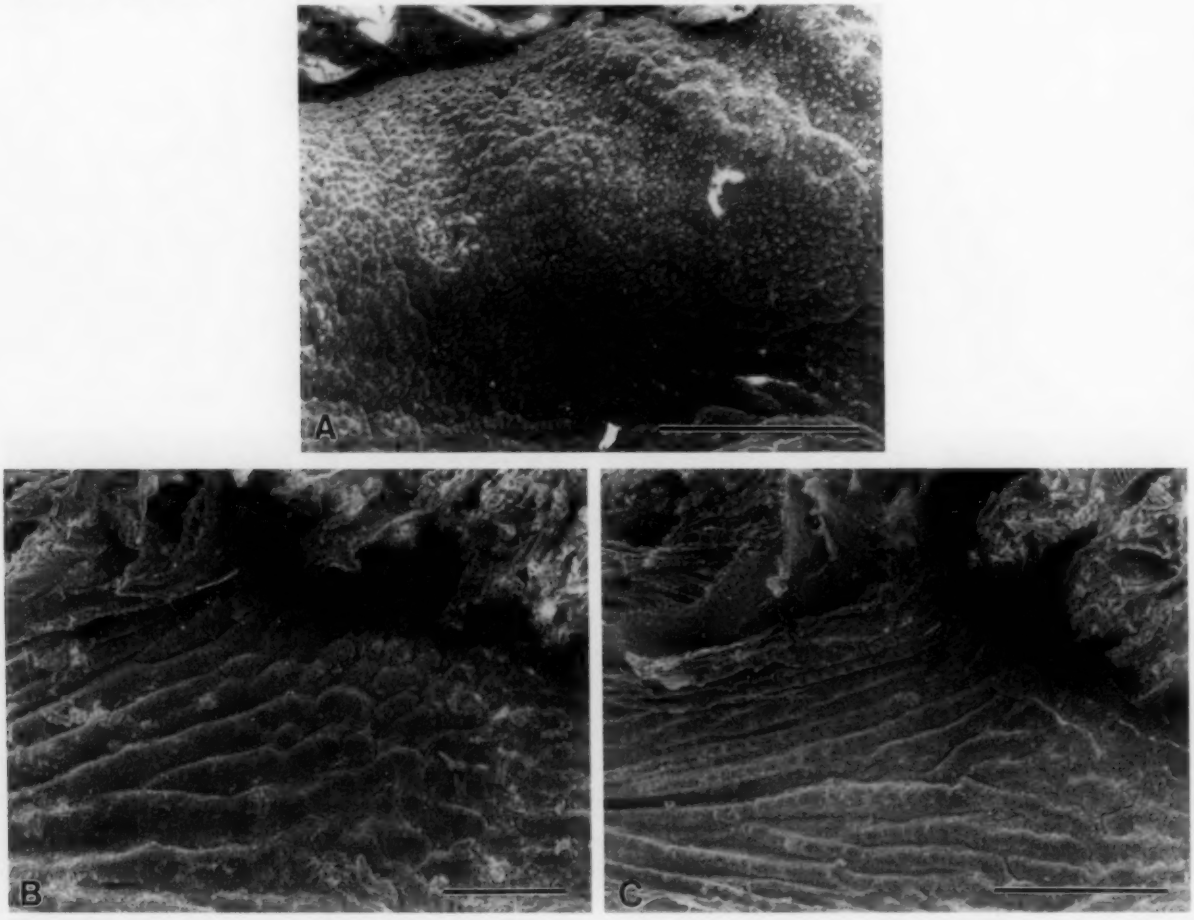
Fig. 4. Scanning electron micrographs of ventral view of the left, lateral part of the palatal organ on the roof of the pharynx. Scale bars = 1 mm. (A) The palatal organ of Sacramento blackfish is highly papillate; shallow indentations for the left branchial arches are at the upper (=lateral) edge of the palatal organ; (B) plicate, nonpapillate palatal organ of Sacramento squawfish, lacking indentations for the branchial arches; (C) the palatal organ of California roach is similar to that of Sacramento squawfish (Fig. 4B).



orders of magnitude more microspheres (596–912 microspheres·individual^{-1}). Suspended particles in the size range of these microspheres (30–90 μm) are common in the aquatic environment (Eisma 1993).

The direct ingestion of water during feeding cannot account for any of the microspheres that were retained by squawfish and roach in the presence of crushed Tetramin, since these species did not feed on the crushed Tetramin. While the direct ingestion of water during particulate feeding on adult *Artemia* could account for some of the microspheres that were observed in the feces, such a process is not sufficient to account for all of the ingested microspheres. For example, a 2.2-g roach specimen retained all of the microspheres (50) in 2 mL of water while feeding on adult *Artemia*. This fish was more likely to have filtered these microspheres from the water rather than to have swallowed a volume of water approximately equal to its body volume (2 mL).

The fish used in this study were not likely to have retained microspheres by drinking water during respiration. While freshwater teleosts do drink small volumes of water (0.6–2.6 μL·g^{-1}·h^{-1} during respiration (Potts et al. 1967; Eddy and Bath 1979)), the squawfish would have had to drink at the rate of at least 150–300 μL·g^{-1}·h^{-1} to ingest three to six microspheres in 10 min.

Unlike blackfish, squawfish and roach retained small microspheres at frequencies significantly greater than their abundance in the water (Fig. 1). Although the small numbers of microspheres retained by squawfish and roach raise questions regarding the biological importance of the differences between the observed and expected size–frequency distributions of microspheres that are described in the Results section, the *G*-tests cannot be ignored because they were corrected for the small sample sizes that were observed in some microsphere size-classes. In addition, the significantly greater retention by squawfish and roach of brine shrimp cysts (210–300 μm) than of microspheres (Table 3) suggests that particle retention by non-suspension-feeding fishes may be influenced by such factors as particle size, surface charge, and (or) texture.

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As an indication of the filtration abilities of these non-suspension-feeding fishes, the data in Table 2 can be used to calculate that, on average during a 10-min period, each squawfish retained the equivalent of all of the brine shrimp cysts present in ~5 mL of water and each roach retained the equivalent of all of the brine shrimp cysts present in ~30 mL of water. These individuals, ranging from 1.8 to 4.9 g, were therefore swallowing all of the brine shrimp cysts from a volume of water equivalent to 1–15 times their body volume during a 10-min period.

Branchial and palatal morphology

By using a fiberoptic endoscope to observe the particle retention process in live blackfish, Sanderson et al. (1991) determined that the gill rakers serve as a barrier that directs particles along the arches to the palatal organ on the roof of the pharynx, where they are retained in mucus aggregates. Particle retention occurs even when blackfish are not feeding, as evidenced by the large numbers of microspheres retained during respiration in the absence of food (Sanderson and Cech 1995).

The branchial and palatal morphology of squawfish and roach is similar whereas blackfish morphology is distinct (Figs. 2, 3, and 4). Unlike the gill rakers of squawfish and roach, the tufted gill rakers of blackfish extend along the arches to the ventral midline of the pharynx. Consequently, more water can exit between the elongate arches on the pharyngeal floor in blackfish than in squawfish and roach. In addition, the tufted, closely spaced gill rakers of blackfish can direct particles along the elongate arches to the lateral sides of the palatal organ whereas the knobby, widely spaced rakers of squawfish and roach are unlikely to serve this purpose. The reduced, exceptionally widely spaced gill rakers on the lateral side of the first branchial arch of squawfish and roach are likely to allow a large volume of water to pass unfiltered between them.

When the mouth is closed, the arches of blackfish fit into grooves along the lateral sides of the palatal organ, dorsal to the pharyngeal floor. This close apposition of the arches and the palatal organ in blackfish could function to direct particles to the palatal organ and result in their transfer from the arches to mucus aggregates on the palatal organ. The numerous papillae on the blackfish palatal organ create a rugose surface that may retain the particle–mucus aggregates. In contrast, the palatal organs of squawfish and roach are neither grooved nor papillate.

The site and mechanism of particle retention by squawfish and roach are not apparent from their branchial and palatal morphology. Conceivably, the brine shrimp cysts (210–300 μm) retained by these species could have been trapped between gill rakers, while the microspheres (31–90 μm) as well as the cysts could have been retained on any sticky buccopharyngeal surface.

Particle retention by benthic feeders

Although the difference between squawfish and roach in the number of brine shrimp cysts retained was not significant ($P = 0.09$, Table 3), the concentration of brine shrimp cysts in the aquarium during the roach experiment was only half that present during the squawfish experiment (Table 2). A lower concentration was used in the roach experiment because the roach, unlike the squawfish, appeared to be agitated at higher concentrations, exhibiting frequent coughs and spits. Northcott et al. (1991) reported "cough-like actions" in *Oreochromis niloticus* (Cichlidae) during suspension feeding on planktonic blue-green algae. They suggested that such regulatory behavior is involved in maintaining constant ingestion rates at high algal densities (Northcott et al. 1991). Due to the lower concentration of cysts in the roach experiment, the number of cysts in the feces of roach was expected to be lower than in the feces of squawfish. However, roach individuals ingested, on average, three times the number of cysts that were ingested by squawfish (Table 2), suggesting that roach may in fact have a higher particle retention rate than squawfish.

The greater number of cysts in the feces of roach was not a result of the roach's higher consumption of *Artemia*. Evidence that the cysts found in the feces were filtered from the water, rather than ingested as a result of their adherence to the *Artemia*, was obtained by comparing the total number of *Artemia* swallowed during the experiment with the total number of cysts ingested during that experiment. After the eight squawfish specimens captured a total of 23 *Artemia*, a total of 909 cysts were counted in the squawfish feces. In contrast, after the eight roach captured a total of 21 *Artemia*, 2854 cysts were counted in the roach feces.

Although the squawfish had significantly greater oral cavity volumes than the roach and the blackfish, they did not retain more microspheres and cysts. As discussed above, the higher retention of cysts by roach than by squawfish, while not statistically significant, is suggestive of substantial particle retention abilities in roach, a benthic-feeding species that does not suspension feed.

Mechanisms of particle retention by non-suspension-feeding fishes

The gill rakers of some cyprinid species may function like a mechanical sieve (e.g., Hoogenboezem et al. 1993; van den Berg et al. 1994*a*, 1994*b*). Mechanisms other than sieving by gill rakers may also be used to separate food particles from water or from a sediment–water slurry. Hlohowskyj et al. (1989) proposed that the unusual pharyngeal papillae of cyprinid benthic feeders in the genus *Hybognathus* may serve in an undetermined manner as an accessory filtering system to the gill rakers. Sibbing and Uribe (1985) and Sibbing (1988) suggested that local muscular bulging of the palatal organ in the suspension-feeding and benthic-feeding cyprinid *Cyprinus carpio* serves to press individual food particles against the floor of the pharynx while repetitive "back-washing" movements resuspend other food and inorganic particles. Eventually, this process is thought to result in the retention of food particles, as inorganic particles are flushed through the branchial slits and out of the opercula (Sibbing et al. 1986).

Mucus is an important component of the particle retention process in many suspension-feeding fishes, serving to retain, aggregate, and (or) transport particles inside the buccopharyngeal cavity (e.g., Hoogenboezem and van den Boogaart 1993; van den Berg et al. 1994*c*; Sanderson et al. 1996). Teleosts in general possess gill rakers and mucus cells in the epithelial lining of the buccopharyngeal cavity (e.g., Walker et al. 1981). Our results suggest that these features may be sufficient for the retention of small particles (31–300 μm) by non-suspension-feeding species. We have demonstrated that the water transport behaviors associated with suspension

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feeding (i.e., ram feeding and repetitive suction) are not necessary for the retention of small particles. Non-suspension-feeding fish species may routinely retain small particles during particulate feeding and respiration.

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Effects of trophic position and lipid on organochlorine concentrations in fishes from subarctic lakes in Yukon Territory

Karen A. Kidd, David W. Schindler, Raymond H. Hesslein, and Derek C.G. Muir

Abstract: Concentrations of organochlorines in lake trout (*Salvelinus namaycush*), burbot (*Lota lota*), and northern pike (*Esox lucius*) from subarctic lakes in Yukon Territory varied significantly among populations, and these differences could not be ascribed to size or age of the fishes. For each species, lipid content and trophic positioning (measured by stable nitrogen isotope ratios; $\delta^{15}N$) were significantly different across populations, and the latter differences could not be attributed to variable $\delta^{15}N$ at the base of the food webs. Across lakes, concentrations of $\sum$PCB, $\sum$DDT, chlorinated bornanes (CHB), chlordane ($\sum$CHL), chlorobenzenes ($\sum$CBZ), and hexachlorocyclohexane ($\sum$HCH) in lake trout and northern pike muscle and burbot liver were significantly related to their $\delta^{15}N$. The slopes of these log organochlorine – $\delta^{15}N$ relations were greatest for more lipophilic contaminants ($\sum$PCB, $\sum$DDT, CHB), indicating that they bioaccumulate to a greater degree than less lipophilic contaminants. Lipid significantly predicted organochlorine concentrations both within and among populations of lake trout, and the slopes did not vary significantly with contaminant lipophilicity. Among-lake differences in $\sum$HCH in trout muscle were removed by adjusting concentrations by the covariate lipid. Lipid-adjusted concentrations of CHB, $\sum$PCB, $\sum$DDT, $\sum$CHL, and $\sum$CBZ in lake trout remained significantly different between lakes, and these differences were attributed to variable food chain lengths.

Résumé : Les concentrations d'organochlorés chez le touladi (*Salvelinus namaycush*), la lotte (*Lota lota*) et le grand brochet (*Esox lucius*) de lacs subarctiques du Yukon variaient de façon importante d'une population à l'autre, et ces différences ne pouvaient être attribuées à la taille ni à l'âge des poissons. Chez chaque espèce, la teneur en lipides et la position trophique (mesurée par les ratios des isotopes stables d'azote; $\delta^{15}N$) étaient significativement différents d'une population à l'autre, et ces dernières différences ne pouvaient être attribuées à la variabilité de $\delta^{15}N$ à la base des réseaux alimentaires. D'un lac à l'autre, les concentrations de $\sum$PCB, de $\sum$DDT, de boranes chlorés (CHB), de chlordane ($\sum$CHL), de chlorobenzènes ($\sum$CBZ) et d'hexachlorocyclohexane ($\sum$HCH) dans le tissu musculaire du touladi et du grand brochet et dans le tissu hépatique de la lotte étaient significativement reliées à leur $\delta^{15}N$. Les pentes de ces relations log organochlorés – $\delta^{15}N$ étaient les plus fortes pour les contaminants plus lipophiles ($\sum$PCB, $\sum$DDT, CHB), ce qui indique que ces derniers se bioaccumulent davantage que les contaminants moins lipophiles. Les lipides étaient significativement corrélés aux concentrations d'organochlorés tant à l'intérieur des populations de touladi que d'une population à l'autre, et les pentes ne variaient pas significativement selon la lipophilie des contaminants. On a pu aplanir les différences inter-lacs des concentrations de $\sum$HCH dans le tissu musculaire du touladi en corrigeant les concentrations en fonction de la covariable lipidique. Les concentrations de CHB, de $\sum$PCB, de $\sum$DDT, de $\sum$CHL et de $\sum$CBZ chez le touladi ajustées en fonction des lipides sont demeurées significativement différentes d'un lac à l'autre, et ces différences ont été attribuées à la variabilité de la longueur des chaînes alimentaires.
[Traduit par la Rédaction]

Introduction

Organochlorines accumulate to a greater extent in fishes (Larsson et al. 1996) and fish tissues (Muir et al. 1990) that are higher in lipid, and this partitioning is directly correlated to the lipophilicity of the contaminants (Mackay 1982). However, concentrations of these pollutants in upper-trophic-level organisms often exceed that expected from lipid content alone (Oliver and Niimi 1988; Swackhamer and Hites 1988). To examine the effects of food-web structure on organochlorine concentrations in top predators, Rasmussen et al. (1990) categorized lake trout (*Salvelinus namaycush*) populations into one of three classes based on the presence–absence of pelagic prey species, with class 1 and 3 populations having the shortest and longest underlying food chains, respectively. Using these discrete groupings, they demonstrated that lake trout from lakes with the longest pelagic food chains had the highest concentrations of PCBs and that both the length of the food chain

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and fish lipid content had significant effects on the organochlorine concentrations in these top predators. After removing the effects of lipid using analysis of covariance (ANCOVA), Bentzen et al. (1996) found that PCB concentrations in lake trout remained significantly different across these classes, which they attributed to differences in food chain length and (or) organochlorine inputs to these systems. While these studies demonstrate that trophic positioning is an important factor in explaining differences in organochlorine concentrations in top predators, it is not possible, with such population-based trophic assignments, to examine the importance of dietary habits on contaminant accumulation in individuals. Such effects may be better understood and quantified if the integer-based assignments are replaced by a trophic continuum. In fact, Vander Zanden and Rasmussen (1996) recently demonstrated that PCB concentrations in lake trout are better predicted by a continuous variable describing trophic positioning than the class assignments used by Rasmussen et al. (1990).

Difficulties in determining an individual's trophic position have often precluded our ability to quantify the effects of food web structure on fish contaminant concentrations. Traditional methods using stomach content analyses are being supplemented or replaced with tissue stable nitrogen isotope ($\delta^{15}N$) analyses. The heavier isotope (^{15}N) increases 3–5‰ (parts per thousand or per mil) from prey to predator (Peterson and Fry 1987), thereby providing a continuous variable with which to determine the relative trophic position of organisms within food webs. Unlike stomach content analyses, which represent recent dietary habits, $\delta^{15}N$ represent the fish's diet over months to years (Hesslein et al. 1993) and provide insight into trophic positioning when stomachs are empty (Kidd 1996). It is only recently that this technique has been used in bioaccumulation studies; concentrations of organochlorines in biota are significantly related to their $\delta^{15}N$ through both freshwater and marine food webs (Broman et al. 1992; Kiriluk et al. 1995; Kidd et al. 1995*a*, 1995*b*; Kucklick et al. 1996).

Measurements of $\delta^{15}N$ in top predators may be used to directly examine the among-system differences in organochlorine bioaccumulation if the $\delta^{15}N$ signals at the base of these food webs are comparable. However, $\delta^{15}N$ in particulate organic matter and in primary consumers varies with the productivity of systems (Gu et al. 1996) and with inputs of sewage (Estep and Vigg 1985; Cabana and Rasmussen 1996), and these basal differences are reflected in the $\delta^{15}N$ of upper trophic level consumers (Minagawa and Wada 1984). In freshwater systems, tissue $\delta^{15}N$ signals of filter-feeding mussels and other primary consumers ranged from 1.2 to 13‰, and some of this variability was attributed to anthropogenic nitrogen inputs to the watersheds of the high-$\delta^{15}N$ systems (asymptotic $\delta^{15}N$ values of primary consumers were 10.9 and 3.3‰ for heavily populated (>100 people/km^2) and less densely populated (1–100 people/km^2) systems, respectively; Cabana and Rasmussen 1996). Two of the three subarctic lakes in this study are largely uninhabited, and the third, Lake Laberge, is located 30 km downstream of the City of Whitehorse (population of approximately 23 000) on the Yukon River system, Yukon Territory. Because of the large size of this lake (volume of 11.1 km^3) and its drainage basin (25 385 km^2; Kidd 1996), we hypothesized that there would be little or no impact of human nitrogen inputs on the basal $\delta^{15}N$ of this lake at these population densities (~1 person/km^2).

In 1990, unusually high concentrations of organochlorines were found in lake trout muscle and burbot (*Lota lota*) liver from Lake Laberge (Kidd et al. 1995*a*). As a result of the high toxaphene (chlorinated bornanes; CHB) concentrations, the commercial, sport, and subsistence fisheries on the lake were closed. Previously, we demonstrated that these fishes contained higher concentrations of CHB because they occupied a significantly higher trophic position, as determined by $\delta^{15}N$, than the same species from the reference lakes (Kidd et al. 1995*a*) and not because of atypical fluxes of these organochlorines to Lake Laberge or unusual bioaccumulation of this contaminant through the food web. Herein, we use $\delta^{15}N$ measurements to examine the relative importance of trophic positioning and lipid content, as well as size and age, on the accumulation of organochlorines in top predators and forage fishes both within and among lakes.

Methods

Lake trout, burbot, northern pike (*Esox lucius*), lake whitefish (*Coregonus clupeaformis*), round whitefish (*Prosopium cylindraceum*), and longnose sucker (*Catostomus catostomus*) were collected under ice in the spring of 1992, and over the summer season in Lake Laberge (61°11′N, 135°12′W) and two reference lakes, Fox (61°14′N, 135°28′W) and Kusawa (60°20′N, 136°22′W), in 1992 and 1993. Physical, limnological, and fish community characteristics of these lakes and methods for fish capture and sampling are detailed in Kidd (1996), Sparling and Connor (1996), and Thompson (1996*a*). Weight, length, sex, and age (except longnose sucker) of each fish was determined. Burbot and northern pike were not netted in Kusawa Lake, and longnose sucker were not netted in Fox Lake. Fishes were sampled for dorsal muscle and liver (burbot only) on site or after being frozen and partially thawed upon return to the laboratory. All samples were kept on ice and frozen a few hours after their collection. To examine among-lake variability in basal $\delta^{15}N$ and determine whether it was valid to use unadjusted $\delta^{15}N$ values of fishes as a direct measure of trophic positioning, mollusks were collected from several locations within each lake between May and August of 1992 through 1994 and included taxa that were commonly found in longnose sucker and round and lake whitefish stomachs (Kidd 1996). Individual gastropods, with the exception of Valvatidae or Planorbidae, were removed from their shells on site and pooled and analysed by family and location. Three families were collected in each lake and included species of Lymnaeidae (*Lymnaea, Fossaria, Bakerilymnaea, Stagnicola*), Planorbidae (*Gyraulus*), and Valvatidae (*Valvata*). These mollusks feed primarily upon periphyton and plant material, although *Lymnaea* are considered to be scavengers of both plant and animal material (Pennak 1953). We made the assumption that the feeding habits of these mollusks would be comparable across the three lakes. As most sampling for this study was done over the summer season and not over an entire year, mollusks, not zooplankton, were chosen to examine $\delta^{15}N$ signals at the base of these food webs, because small planktonic invertebrates exhibit greater temporal variability in their tissue $\delta^{15}N$ than larger macroinvertebrates (Cabana and Rasmussen 1996). In addition, zooplankton tows done in early August indicated that 70, 34, and 40% of the individuals in the zooplankton samples from Laberge, Kusawa, and Fox lakes, respectively, were the predaceous cyclopoid *Cyclops scutifer*. Unlike Laberge, zooplankton samples from Kusawa and Fox lakes were equally dominated by the herbivorous cladoceran *Holopedium gibberum* or calanoid *Diaptomus sicilis*, respectively (A. Salki, Department of Fisheries and Oceans, Winnipeg, Man., personal communication). These community differences precluded using bulk zooplankton $\delta^{15}N$ to examine differences in basal $\delta^{15}N$ among lakes.

For stable isotope analyses, skinless dorsal muscle tissue from

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individual fishes and whole mollusks (pretreated with 1 M HCl to dissolve any remaining shells) were dried in an oven at 60°C and ground to a fine powder. Initially, samples were analysed by vacuum sealing approximately 15 mg of dried tissue in a Vycor tube with 1 g copper wire, 1 g copper oxide wire, and a 1-mm^2 piece of silver foil. These tubes were combusted at 850°C for 4 h and then at 600°C for 2 h. Nitrogen gas was cryogenically separated from CO_2, trapped in a molecular sieve, and analysed on a dual-inlet isotope ratio mass spectrometer (VG Micromass 602E). Most tissue samples were analysed using the following fully automated method. Dried samples of 1–4 mg were combusted in a Carlo Erba NA1500 elemental analyzer. Sample gases were introduced into a VG Optima automated mass spectrometer with helium carrier gas, and water and CO_2 were cryogenically removed using magnesium perchlorate and an Ascarite® column, respectively. All samples were standardized against N_2 in air as follows:

$$\delta N‰ = ((R_{sample}/R_{standard}) - 1) \times 1000$$

where $R = {}^{15}N/{}^{14}N$. A laboratory working standard of Pharmamedium was run with every 10 samples. In this study, mollusk data are presented for the three lakes as a mean $\delta^{15}N$ value for all families analysed. Precision of these instruments for nitrogen isotope analysis over several years of use has been 0.4‰ (2 SD). Replicate analyses of 35 samples resulted in an average absolute deviation about the mean of 0.22‰.

Whenever possible, fish ranging in size and an equal number of males and females were chosen for organochlorine analyses. Fish muscle (skin included) and whole round whitefish were homogenized with dry ice. For Lake Laberge only, round whitefish were analyzed as composite samples of four to six individuals grouped by weight and location. Five to 10 g of ground tissues were combined with anhydrous Na_2SO_4 (heated at 600°C for 16 h prior to use), and internal standards of aldrin (or PCB30) and octachloronaphthalene (OCN) were added to the samples prior to extraction. Burbot liver tissues were extracted two times with hexane using a ball mill. Muscle and whole-body samples were Soxhlet extracted for 4 h using dichloromethane (DCM) – hexane (1:1). Extractable lipids were determined gravimetrically with 1/10th of the extract. After removing the remaining lipids using automated gel permeation chromatography on SX-3 Biobeads with DCM–hexane as the eluant, the extract was separated on Florisil (8 g; 1.2% deactivated with HPLC-grade water) into three fractions: hexane (F1), hexane–DCM (85:15) (F2), and hexane–DCM (1:1) (F3). F1 contained all PCBs and chlorobenzenes; and F2 contained most hexachlorocyclohexane, chlorinated bornane, chlordane, and DDT congeners. Both fractions were analysed for organochlorines using a Varian 6000 or 3600 gas chromatograph with a ^{63}Ni-electron capture detector and a 60 m by 0.25 mm i.d. DB-5 column (film thickness 0.25 µm; J & W Scientific) with H_2 carrier gas (approximately 1 mL/min; initial flow of 2.0 mL/min; constant pressure of 22.5 psi; 1 psi = 6.895 kPa) and N_2 makeup gas. The column temperature started at 100°C, increased 15°C/min to 150°C, 3°C/min to 265°C, and then was maintained at this temperature for the remainder of the 57-min run. OCN had a retention time of 47.7 min under these conditions. Samples were quantified against external standards that were obtained from National Research Council of Canada (Halifax, N.S.), Ultra Scientific (Hope, R.I.), National Institute of Standards and Technology (NIST; Gaithersburg, Md.), and Cambridge Isotope Laboratories (Cambridge, Mass.).

Minimum detectable levels (MDL) were calculated as described in Long and Winefordner (1983). MDLs averaged 0.02 ng/g for individual tri- to octa-chlorobiphenyls and most organochlorine pesticides. Average recoveries of internal standards for all samples were 80 and 89% for F1 (PCB30 or aldrin) and F2 (OCN), respectively. The laboratory successfully participated in a number of interlaboratory studies quantifying organochlorines in biological tissues during the time period that these samples were analyzed. NIST 1588 cod liver was used as a reference material.

Results were examined for six major contaminant groups that represented a range of lipophilicities. Hexachlorocyclohexane (∑HCH) was quantified as the sum of αHCH, βHCH, and γHCH, which have log K_{ow}s of 3.7–3.8 (de Bruijn et al. 1989). The chlorobenzenes (∑CBZ) detected in these fishes included 1,2,3,4-tetrachlorobenzene, 1,2,4,5-tetrachlorobenzene, hexachlorobenzene, and pentachlorobenzene (log K_{ow}s from 4.5 to 5.5; Mackay et al. 1992). Chlordane-related compounds were quantified with individual standards except for Cl_9 components, nonachlor III, and C5, which were quantified using the response factor (RF) of *trans*-nonachlor, and the Cl_{7-8}-containing components (see Dearth and Hites 1991 for nomenclature) "C," C1, C2, and C4, which were quantified using the RF of *trans*-chlordane. Total chlordane (∑CHL) was the sum of all chlordane-related compounds including heptachlor epoxide. An average log K_{ow} of 5.54 was assumed for chlordane (Howard 1991). ∑DDT congeners included *p,p′*- and *o,p′*-DDT, *p,p′*- and *o,p′*-DDE, and *p,p′*- and *o,p′*-DDD (log K_{ow}s from 6.2 to 6.9 for *p,p′*-DDD, DDE, and DDT; de Bruijn et al. 1989). Total PCBs (∑PCB) were quantified as the sum of all congeners (see Table 1 for complete list), and concentrations in fishes consisted mainly of penta-, hexa-, and hepta-chlorinated PCBs that range in log K_{ow} from 6.2 to 7.0 (Mackay et al. 1992). Chlorinated bornanes (toxaphene; CHB) were quantified using a single response factor based on the areas of 20 peaks in the standard (Muir et al. 1990). Although CHB in environmental samples can differ considerably in their patterns of CHB when compared with technical toxaphene (Stern et al. 1992; Miskimmin et al. 1995), the octanol–water partition coefficients for the environmentally relevant individual congeners have not yet been determined. For the general comparative purposes of this study, the log K_{ow} of technical toxaphene (6.44) was used (Saleh 1991). In general, the lipophilicity of the major contaminant groups in this study are as follows: ∑DDT ≈ ∑PCB ≈ CHB > ∑CHL > ∑CBZ > ∑HCH.

The Statistical Analysis Systems (SAS 1985) general linear models (GLM) procedure, and Duncan's multiple range or Student's *t* tests were used to determine if significant among-lake differences existed in organochlorine concentrations, weight, length, age, percent lipid, or $\delta^{15}N$ for each species. Variables were log transformed if means were proportional to standard deviations and if the transformation reduced the heterogeneity of variances; lipid was not arcsine transformed as is suggested for percentages or proportions as this transformation did not reduce the skewness in the data. Within-lake relations between organochlorine concentrations and $\delta^{15}N$, lipid, size, or age were examined for each species using GLM simple least-squares linear regressions. Analysis of covariance (ANCOVA) was used to examine whether the slopes of the organochlorine–lipid or organochlorine–$\delta^{15}N$ relations were the same across lakes. If no significant interaction was observed in the lake × lipid or lake × $\delta^{15}N$ terms in these models, indicating homogeneity of slopes, and the covariate remained significant after the interaction term was removed from the model, the covariate-adjusted least-squares mean and individual fish organochlorine concentrations were calculated (Hebert and Keenleyside 1995). These covariate-adjusted means were compared among lakes using Tukey tests. Data were pooled across lakes only if the within-lake slopes were homogenous as described above, and then the slopes of the general log organochlorine – lipid or log organochlorine – $\delta^{15}N$ relations were calculated and compared across major organochlorine groups using ANCOVA and Tukey tests. Multiple regression analyses was conducted to determine which combination of variables (lipid, length (or weight), $\delta^{15}N$, or age) best predicted the concentrations of ∑HCH, ∑CBZ, ∑CHL, ∑DDT, ∑PCB, and CHB in each species across all lakes. Measurements that were highly correlated, such as length and weight, were not included in the same regression model, and variables were retained in the model only if they explained a significant proportion of the variability over and above that explained by other variables (type III sums of squares in SAS). Multiple regression models with the largest coefficients of determination were retained as the best predictors of fish organochlorine

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Table 1. Mean ± SD (range) of ∑CBZ, ∑HCH, ∑CHL, ∑PCB, ∑DDT, and CHB concentrations (ng/g wet weight) in fish muscle and burbot liver collected from Fox, Kusawa, and Laberge lakes, Yukon Territory.

	n	∑CBZ	∑HCH	∑CHL	∑PCB*	∑DDT	CHB
Fox Lake							
Burbot	14	6.95±2.59*a*	14.3±4.66*a*	19.0±7.67*a*	45.9±23.8*a*	67.7±30.5*a*	68.4±23.5*a*
		(12.9–4.12)	(24.4–6.29)	(38.9–11.4)	(103–18.7)	(125–32.7)	(109–35.4)
Males	8			16±6.2			
Females	6			23±7.9			
Lake trout	16	0.5±0.6*a*	0.8±1*a*	1.8±1.9*a*	5.34±3.57*a*	6.00±5.01*a*	7.2±7.3*a*
		(2.48–0.07)	(4.3–0.05)	(7.95–0.19)	(14.3–1.35)	(18.9–1.38)	(27.3–0.65)
Lake whitefish	13	0.40±0.36*a*	0.4±0.3*a*	0.65±0.50*a*	3.75±4.10*a*	4.3±3.6*a*	4.2±4.5*a*
		(1.36–0.12)	(0.98–<0.01)	(1.88–0.15)	(16.3–1.10)	(14.1–0.81)	(18.5–0.33)
Northern pike	8	0.2±0.1*a*	0.09±0.04*a*	0.1±0.07*a*	0.4±0.3*a*	0.54±0.31*a*	0.33±0.64*a*
		(0.31–0.06)	(0.17–0.05)	(0.28–0.05)	(0.95–0.09)	(1.05–0.16)	(1.96–<0.01)
Males	5	0.08±0.03					
Females	3	0.12±0.04					
Round whitefish	8	0.46±0.21*a*	0.69±0.35*a*	0.4±0.2*a*	1.1±0.23*a*	0.90±0.42*a*	1.3±0.71*a*
		(0.78–0.22)	(1.50–0.36)	(0.64–0.07)	(1.51–0.85)	(1.32–0.20)	(3.44–0.74)
Kusawa Lake							
Lake trout	10	1.2±0.87*b*	1.2±1.2*a*	17.3±8.81*b*	85.4±82.5*b*	44.2±68.0*b*	121±78.9*b*
		(2.96–0.11)	(4.14–0.18)	(35.7–7.02)	(311–28.9)	(228–9.20)	(235–22.9)
Lake whitefish	12	0.56±0.31*a*	0.47±0.36*a*	2.0±1.7*b*	19.6±20.9*b*	15.1±17.3*b*	14.7±12.6*b*
		(1.22–0.16)	(1.12–0.12)	(6.48–0.44)	(69.1–2.17)	(59.4–1.09)	(54.9–2.72)
Longnose sucker	6	0.29±0.10*a*	0.34±0.081*a*	1.6±0.53*a*	10.5±5.92*a*	10.3±3.59*a*	7.08±3.08*a*
		(0.42–0.13)	(0.48–0.25)	(2.27–0.91)	(18.5–4.29)	(13.8–5.54)	(15.7–4.88)
Males	3	0.28±0.03					
Females	3	0.40± 0.07					
Round whitefish	6	0.62±0.12*a*	0.67±0.20*a*	1.7±0.88*b*	2.26±0.71*b*	1.3±0.52*a*	13.4±8.76*b*
		(0.79–0.46)	(0.93–0.46)	(3.11–0.58)	(3.07–1.10)	(2.09–0.56)	(24.1–2.13)
Lake Laberge							
Burbot	16	19.8±8.23*b*	26.1±6.80*b*	261±201*b*	1210±926*b*	2530±1990*b*	2170±1130*b*
		(40.4–10.0)	(37.9–14.6)	(681–87.7)	(3640–485)	(7330–901)	(4470–879)
Lake trout	12	3.01±1.33*c*	3.41±1.69*b*	44.3±48.0*c*	453±832*c*	527±907*c*	387±415*c*
		(5.47–1.44)	(6.96–1.40)	(166–13.3)	(2530–53.6)	(3010–44.6)	(1440–112)
Lake whitefish	30	1.1±0.59*b*	1.6±1.0*b*	8.91±5.65*c*	66.3±57.1*c*	82.5±63.6*c*	58.7±47.5*c*
		(3.30–0.13)	(5.68–0.20)	(28.7–1.78)	(278–6.57)	(284–10.4)	(201–5.53)
Longnose sucker	12	0.3±0.2*a*	0.37±0.18*a*	2.1±1.4*a*	13.4±7.34*a*	16.1±7.77*a*	13.2±10.4*a*
		(0.59–0.6)	(0.71–0.14)	(5.17–0.27)	(23.9–1.09)	(26.4–2.88)	(35.2–1.8)
Northern pike	8	0.2±0.06*a*	0.1±0.04*a*	0.94±0.31*b*	7.20±3.12*b*	8.35±2.74*b*	8.61±3.64*b*
		(0.26–0.08)	(0.18–0.07)	(1.42–0.50)	(12.0–3.06)	(12.3–5.46)	(11.4–3.9)
Round whitefish**	7	0.77±0.48*a*	0.92±0.43*a*	3.2±2.0*b*	17.0±9.67*c*	22.6±11.0*b*	21.2±9.73*b*
		(1.67–0.22)	(1.39–0.32)	(5.67–0.84)	(28.5–6.00)	(33.8–7.01)	(33.8–3.98)

Note: Within species and contaminants, values among lakes with different letters were significantly different. Means for males and females are shown separately when significant between-sex differences were found.

*Sum of CB 4/10, 7, 6, 8/5, 19, 18, 17, 24/27, 16/32, 26, 25, 31, 28, 33, 22, 45, 46, 52, 49, 47, 48, 44, 42, 47/71, 64, 40, 74, 70/76, 95, 56/60, 91, 84/89, 101, 99, 83, 97, 87, 85, 136, 110, 82, 151, 144/135, 149, 118, 134, 114, 131, 146, 153, 132, 105, 141, 130/176, 179, 137, 138, 158, 178/129, 175, 187, 183, 128, 185, 174, 177, 171, 156, 201/157, 172/197, 180, 193, 191, 200, 170, 190, 198, 199, 196/203, 189, 208, 195, 207, 184, 206, 209.

**Composite samples of whole fish grouped by size.

concentrations if they predicted more of the variability than any single variable, and plots of the residuals were examined to ensure that the assumptions of independence and homogeneity of variances were met.

Results

Concentrations of organochlorines in fishes were generally highest in Lake Laberge, midrange in Kusawa Lake, and lowest in Fox Lake (Table 1), and these differences could not be ascribed to differences in size or age of these populations (Table 2). Within each lake and species, organochlorine data for males and females were pooled because few significant sex-related differences were observed (see Table 1 for exceptions). Both percent lipid and $\delta^{15}N$ of the top predators, lake trout, burbot, and northern pike (Laberge > Kusawa > Fox), and the forage fish, lake whitefish (Laberge > Kusawa, Fox), were significantly different among lakes (Table 2), and the latter differences were not due to variable $\delta^{15}N$ at the base of the food webs (Fig. 1). Only within-species relations were examined herein, because the same tissues were not analysed for organochlorines for all fishes in this study, and differences in

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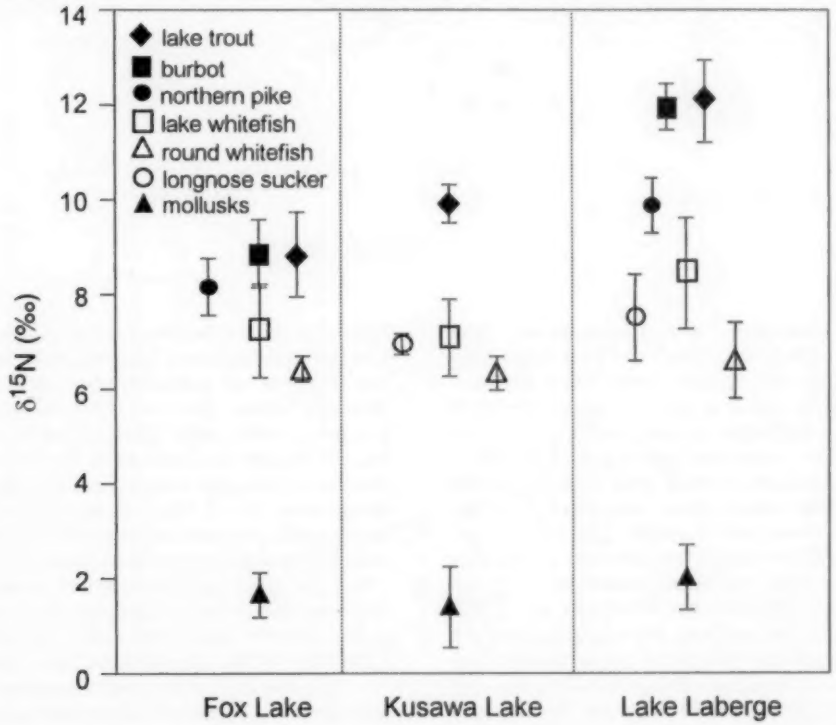
Table 2. Mean (±SD) $\delta^{15}N$ and percent lipid of fish muscle (liver for lipid content in burbot) and weight, length, and age of fishes from Laberge, Fox, and Kusawa lakes, Yukon Territory.

Species	Lake	*n*	$\delta^{15}N$ (‰)	% Lipid	Length (mm)	Weight (g)	Age (years)
Burbot	Laberge	16	12.0±0.48*a*	38.55±15.38*a*	548±132*a*	1370±800*a*	10±5*a*
	Fox	14	8.86±0.71*b*	28.49±10.21*b*	554±87.7*a*	1350±558*a*	10±3*a*
Lake trout	Laberge	12	12.1±0.87*a*	6.77±3.17*a*	489±60.4*ab*	1430±586*a*	20±5*a*
	Kusawa	10	9.90±0.40*b*	1.76±1.56*b*	525±90.3*a*	1700±1060*a*	20±7*a*
	Fox	16	8.84±0.89*c*	1.38±1.34*b*	434±87.2*a*	967±626*a*	20±5*a*
Lake whitefish	Laberge	30	8.35±1.1*a*	2.84±1.40*a*	318±81.4*a*	432±251*a*	10±4*a*
	Kusawa	12	7.23±0.65*b*	1.4±0.77*b*	379±32.1*b*	592±156*a*	10±2*a*
	Fox	13	7.22±0.98*b*	0.99±0.66*b*	422±46.0*b*	846±249*b*	10±6*a*
Longnose sucker	Laberge	12	7.51±0.91*a*	0.96±0.51*a*	433±20.8*a*	852±112*a*	—
	Kusawa	6	6.94±0.22*a*	1.2±0.36*a*	419±20.4*a*	767±40.8*a*	—
Northern pike	Laberge	8	9.88±0.58*a*	0.41±0.09*a*	634±77.6*a*	1825±727.5*a*	7±2*a*
	Fox	8	8.16±2.03*b*	0.28±0.09*b*	646±105*a*	2170±957*a*	8±2*a*
Round whitefish	Laberge	7	6.60±0.80*a*	2.1±0.57*a*	221±115*a*	170±280*a*	—
	Kusawa	6	6.32±0.36*a*	2.3±0.53*a*	184±44.8*a*	58±41*a*	4±1*a*
	Fox	8	6.40±1.66*a*	1.8±0.81*a*	244±88.1*a*	180±180*a*	5±3*a*

Note: For each species, values among lakes with different letters are significantly different.

Fig. 1. Mean $\delta^{15}N$ (±SD) of lake trout, burbot, northern pike, lake and round whitefish, and longnose sucker muscle and whole mollusks ($n = 6$ for each lake) from Fox, Kusawa, and Laberge lakes, Yukon Territory (see Table 2 for *n* of fishes).



contaminant and lipid deposition among tissues would likely confound any interpretation of the relative importance of lipid and $\delta^{15}N$ on organochlorine accumulation across species. Although no relation existed within lakes for these two variables, lipid and $\delta^{15}N$ were correlated in lake trout ($r^2 = 0.42$), burbot ($r^2 = 0.24$), and lake whitefish ($r^2 = 0.23$) when all data were pooled.

Mollusk $\delta^{15}N$ was not significantly different across lakes ($P = 0.31$; mean ± SD for Laberge, 2.02 ± 0.69‰; Kusawa, 1.39 ± 0.85‰; Fox, 1.65 ± 0.47‰; Fig. 1). No significant differences in $\delta^{15}N$ were found for all other macroinvertebrates collected from these lakes (Trichoptera (Limnephilidae and Limnephilinae) from Laberge, 2.55 ± 1.38‰ ($n = 9$); Kusawa, 1.62‰ ($n = 2$); Fox, 2.53 ± 1.26‰ ($n = 6$); chironomids

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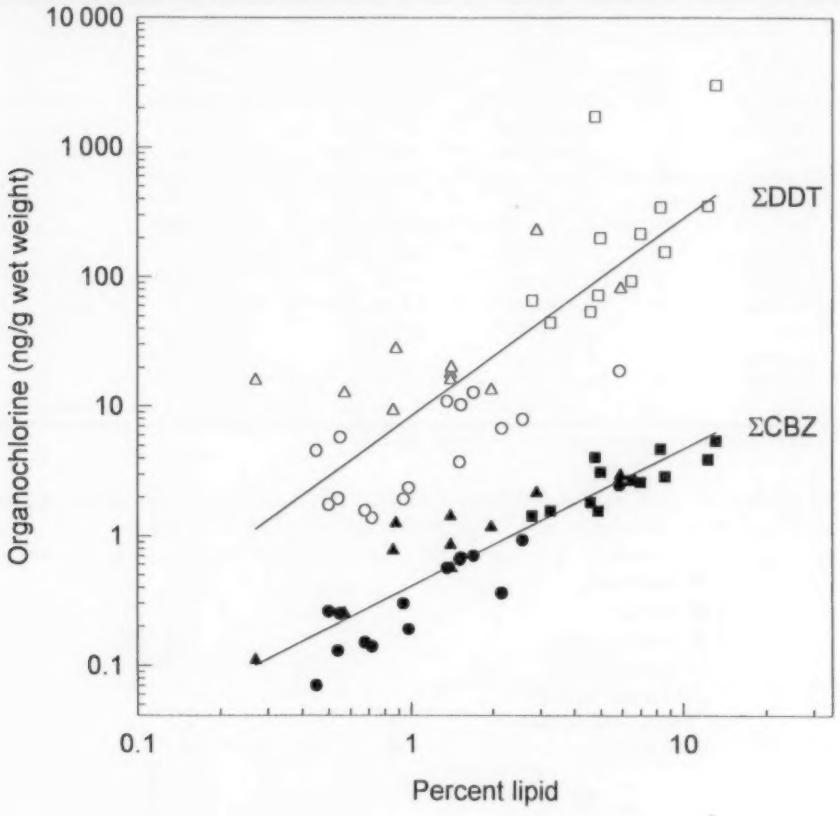
Fig. 2. Relation between log-transformed ∑CBZ or ∑DDT (ng/g wet weight) and log-transformed lipid for all lake trout from Laberge (squares), Fox (circles), and Kusawa (triangles) lakes. Slopes of least-squares linear regression equations are given in Table 3.



(Tanypodinae, Prodiamesinae and Chironominae) from Laberge, 4.02 ± 1.14‰ (n = 8); Kusawa, 4.08 ± 0.62‰ (n = 5); Fox, 4.06 ± 0.97‰ (n = 4); Kidd 1996). These results indicated that basal $\delta^{15}N$ signals in these systems were similar and that fish $\delta^{15}N$ could be directly compared across systems.

Lipid was the only variable that significantly predicted organochlorine concentrations in lake trout both within and among lakes. For Lake Laberge trout, concentrations of log-transformed ∑HCH (P = 0.005, r^2 = 0.56), ∑CBZ (P = 0.001, r^2 = 0.64), and ∑DDT (P = 0.03, r^2 = 0.39) were significantly related to log lipid, while less of the variability in ∑CHL (P = 0.07, r^2 = 0.28), CHB (P = 0.1, r^2 = 0.25), and ∑PCB (P = 0.19, r^2 = 0.17) concentrations was explained by lipid. Lipid was also a significant predictor of organochlorine concentrations in lake trout from Fox (all log organochlorine – log lipid relations were significant at P < 0.01) and Kusawa lakes (all log organochlorine – log lipid relations except ∑PCB were significant at P < 0.03; within-lake relations not shown) and for all lake trout data from the three lakes (Fig. 2, Table 3). The slopes of these log organochlorine – log lipid relations were homogeneous, as determined by the lake × log lipid interaction term in ANCOVA, for all contaminant groups both within (Laberge, P = 0.24; Fox, P = 0.78; Kusawa, P = 0.15) and among lakes (P = 0.24). For this species, lipid was a better predictor of the less lipophilic (∑HCH and ∑CBZ) than more lipophilic organochlorines quantified herein (Table 3). Data were not expressed on a lipid weight basis, because the relations between the untransformed concentrations of organochlorines versus lipid were not isometric (Packard and Boardman 1988). After adjusting organochlorine concentrations for the effects of lipid using ANCOVA, ∑HCH concentrations in lake trout muscle were not significantly different among lakes (P = 0.14), and concentrations of all other organochlorine groups were comparable in Laberge and Kusawa lakes but remained significantly lower in Fox Lake.

For the other species examined herein, log-transformed concentrations of some organochlorines were significantly related to percent lipid when data were pooled across lakes. ∑DDT and ∑CHL were related to lipid content in pike muscle (r^2 = 0.28 and 0.31, respectively; equations not shown); concentrations of all organochlorines were related to lipid content in lake whitefish muscle (r^2 = 0.25–0.63; Table 3); CHB, ∑CBZ, ∑CHL, and ∑HCH concentrations were related to lipid content in round whitefish muscle (r^2 = 0.28–0.42; Table 3); and ∑HCH, ∑CHL, ∑CBZ, and CHB were related to lipid content in longnose sucker muscle (r^2 = 0.22–0.60; Table 3). No such relations were found for burbot. As was observed for lake trout, the slopes of these log organochlorine – lipid relations were not significantly different among major contaminant groups for each species (Table 3). Although lipid

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Table 3. Slopes and r^2 (given in parentheses) from least-squares regression equations of log-transformed organochlorine concentrations versus lipid or $\delta^{15}N$ for all lake trout, burbot, northern pike, lake or round whitefish, and longnose sucker from Laberge, Kusawa, and Fox lakes.

Species		∑HCH	∑CBZ	∑CHL	∑DDT	∑PCB	CHB
Lake trout	log lipid	1.00±0.08*a*	1.06±0.08*a*	1.15±0.2*a*	1.53±0.2*a*	1.18±0.2*a*	1.43±0.2*a*
		(0.83)	(0.84)	(0.49)	(0.66)	(0.43)	(0.52)
	$\delta^{15}N$	0.17±0.04*a*	0.21±0.04*ab*	0.32±0.06*ab*	0.40±0.06*b*	0.36±0.06*ab*	0.40±0.07*b*
		(0.30)	(0.40)	(0.47)	(0.58)	(0.51)	(0.50)
Burbot	$\delta^{15}N$	0.078±0.02*a*	0.13±0.02*a*	0.30±0.03*b*	0.43±0.04*c*	0.40±0.04*bc*	0.42±0.03*bc*
		(0.48)	(0.65)	(0.73)	(0.82)	(0.82)	(0.85)
Northern pike	$\delta^{15}N$	0.077±0.03*a*		0.38±0.08*ab*	0.52±0.09*b*	0.58±0.09*b*	0.59±0.70*b*
		(0.28)		(0.62)	(0.67)	(0.73)	(0.70)
Lake whitefish	Lipid	0.15±0.03*a*	0.13±0.02*a*	0.19±0.04*a*	0.21±0.06*a*	0.17±0.05*a*	0.21±0.04*a*
		(0.45)	(0.45)	(0.37)	(0.29)	(0.24)	(0.38)
	$\delta^{15}N$	0.15±0.05*a*	0.11±0.04*a*	0.27±0.07*a*	0.33±0.08*a*	0.27±0.07*a*	0.30±0.07*a*
		(0.15)	(0.12)	(0.26)	(0.28)	(0.22)	(0.30)
Round whitefish	Lipid	0.15±0.05*a*	0.21±0.06*a*	0.37±0.14*a*			0.44±0.2*a*
		(0.32)	(0.42)	(0.28)			(0.28)
Longnose sucker	Lipid	0.26±0.08*a*	0.41±0.08*a*	0.27±0.13*a*			0.33±0.14*a*
		(0.39)	(0.60)	(0.23)			(0.26)

Note: Values within species for log organochlorine – lipid or log organochlorine – $\delta^{15}N$ slopes with different letters are significantly different.

Fig. 3. Relation between log-transformed ∑CBZ or ∑DDT (ng/g wet weight) and $\delta^{15}N$ for lake trout from Laberge (squares), Fox (circles), and Kusawa (triangles) lakes. Slopes of least-squares linear regression equations and significant differences in slopes are given in Table 3.

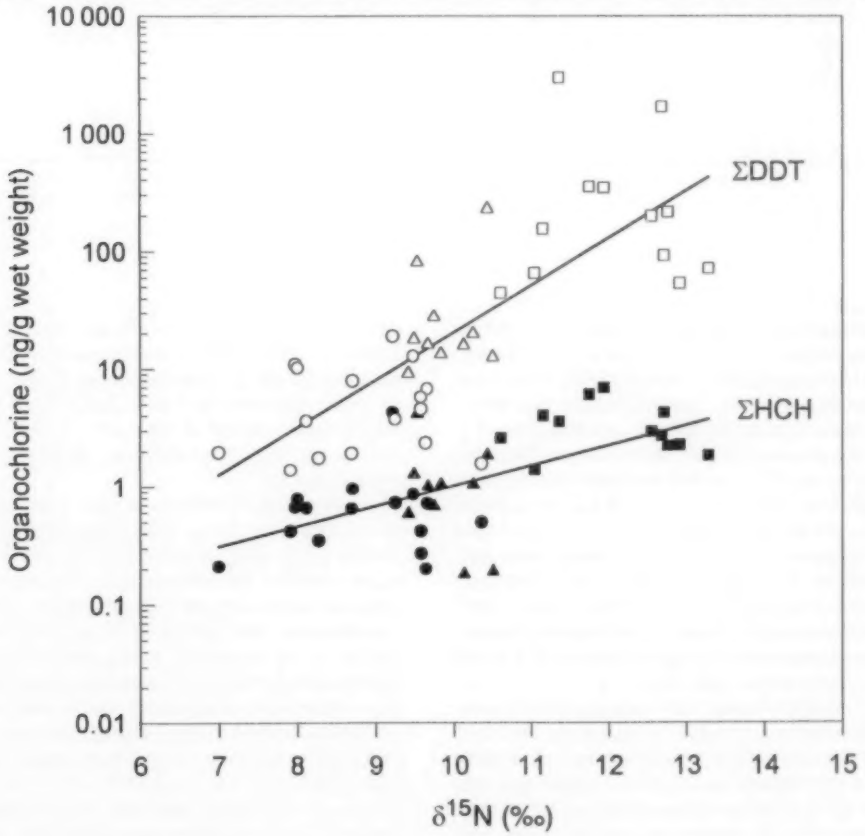

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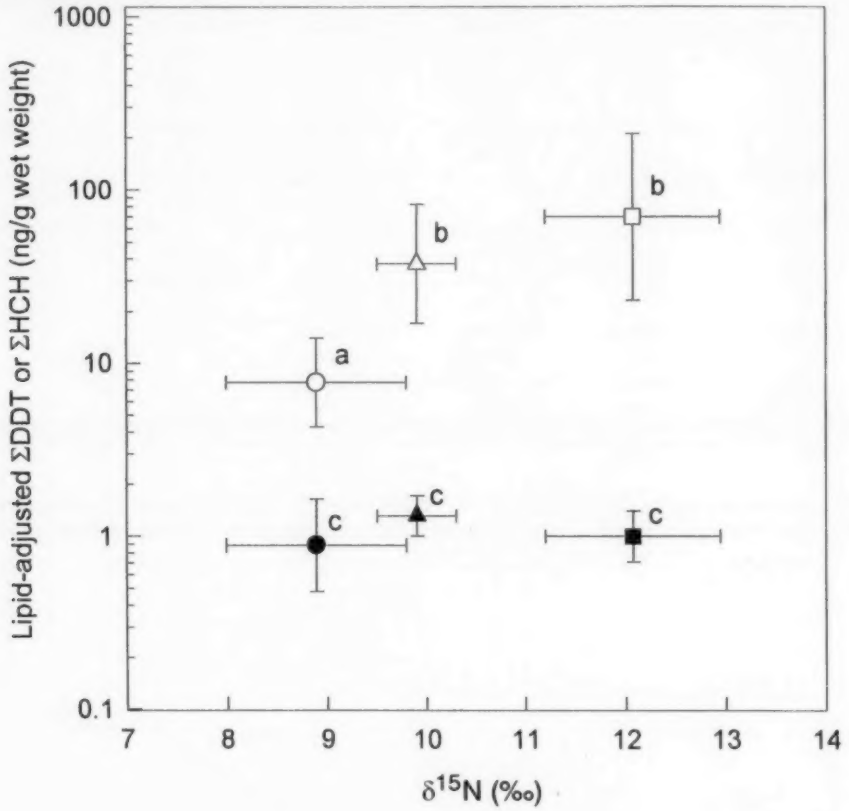
Fig. 4. Means (±SD) of lipid-adjusted ∑HCH (solid symbols) and ∑DDT (open symbols) (geometric means; ng/g wet weight) versus $\delta^{15}N$ (arithmetic means ± SD; ‰) in lake trout muscle from Laberge (squares), Fox (circles), and Kusawa (triangles) lakes. Significant among-lake differences in mean concentrations of these organochlorines are indicated by different letters.



content was significantly different among populations of burbot, northern pike, and lake whitefish (Table 2), and was significantly related to organochlorine concentrations in the latter two species, it could not be used as a covariate to remove among-lake differences in organochlorine concentrations.

In contrast to the organochlorine–lipid relations discussed above, there were no significant relations between concentrations of organochlorines and $\delta^{15}N$ within each lake trout population. However, when trout data from all lakes were combined, the log organochlorine – $\delta^{15}N$ relations were significant and explained 30–58% of the variability in the contaminant data (Fig. 3, Table 3). The slopes of these regressions increased with increasing lipophilicity of the organochlorines and were significantly greater for the more lipophilic CHB and ∑DDT than the less lipophilic ∑HCH.

$\delta^{15}N$ was not a significant predictor of organochlorine concentrations within other fish populations from these lakes and could not be used as a covariate to remove among-lake effects in organochlorine concentrations. However, significant log organochlorine – $\delta^{15}N$ relations were observed when data were pooled across lakes for burbot and northern pike, and these results mirrored those for lake trout; $\delta^{15}N$ was a significant predictor of all contaminant concentrations in burbot liver (r^2 = 0.48–0.85) and all organochlorines except ∑CBZ in northern pike (r^2 = 0.28–0.70), and both the slopes and coefficients of determination were greater for the more lipophilic than less lipophilic organochlorines (Table 3). Concentrations of organochlorines were also significantly related to $\delta^{15}N$ of lake whitefish across all lakes (r^2 = 0.15–0.30), but no differences in the log organochlorine – $\delta^{15}N$ slopes were observed for this species.

Among-lake differences in lake trout organochlorine concentrations were due to both differences in lipid content and trophic positioning of these populations. After adjusting all organochlorine concentrations in lake trout by the covariate lipid, significant among-lake differences remained for all organochlorines but ∑HCH. $\delta^{15}N$ explained a significant proportion of the variability in organochlorine data (except for ∑HCH and ∑CBZ) over and above that explained by lipid when included in multiple regression analyses in place of the categorical variable "lake." This combination of lipid and $\delta^{15}N$ explained more of the variability in organochlorine concentrations (∑DDT, r^2 = 0.75; ∑PCB, r^2 = 0.56; CHB, r^2 = 0.62; ∑CHL, r^2 = 0.58; relations not shown) than either variable alone (Table 3). In addition, the ANCOVA lipid-adjusted concentrations of the more lipophilic organochlorines were significantly related to $\delta^{15}N$ for individuals across the three lakes ($P < 0.005$, n = 37) as follows:

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Table 4. Coefficients and intercepts (±SE) of the multiple regression models predicting ($P < 0.01$) the log-transformed wet weight concentrations of ∑HCH, ∑CBZ, ∑CHL, ∑PCB, ∑DDT, and CHB in lake trout, burbot (liver analysed for organochlorines), northern pike, lake whitefish, and longnose sucker from Laberge, Fox, and Kusawa lakes, Yukon Territory.

	% Lipid	Weight	$\delta^{15}N$	Age	Intercept	r^2	SE_{est}
Lake trout ($n = 37$)[a]							
∑HCH	0.10±0.015	0.51±0.18	—	—	−1.9±0.53*	0.70	0.19
∑CBZ	0.11±0.017	0.52±0.19	—	—	−2.0±0.57*	0.69	0.19
∑CHL	0.051±0.028	1.1±0.27	0.20±0.055	—	−4.8±0.94*	0.71	0.41
CHB	0.060±0.035	1.2±0.33	0.26±0.068	—	−5.0±1.2*	0.70	0.49
∑DDT	0.12±0.025	0.82±0.24	0.22±0.048	—	−3.7±0.82*	0.83	0.38
∑PCB		1.2±0.29	0.31±0.051	—	−5.3±0.93*	0.66	0.48
Burbot ($n = 29$)[b]							
∑HCH	$5.0 \times 10^{-3} \pm 1.9 \times 10^{-3}$	$1.0 \times 10^{-4} \pm 2.8 \times 10^{-5}$	0.057±0.013		0.37±0.12	0.73	0.09
∑CBZ			0.13±0.017	0.59±0.18	−0.95±0.27*	0.72	0.15
∑CHL			0.31±0.028	1.27±0.30	−2.74±0.44*	0.84	0.25
CHB			0.43±0.029	1.09±0.31	−3.07±0.45*	0.90	0.26
∑DDT		$3.1 \times 10^{-4} \pm 7.6 \times 10^{-5}$	0.44±0.031		−2.45±0.35*	0.89	0.27
∑PCB		$2.5 \times 10^{-5} \pm 7.5 \times 10^{-5}$	0.40±0.031		−2.24±0.35*	0.87	0.28
Northern pike ($n = 16$)							
∑CHL	1.96±0.56		0.35±0.059		−4.33±0.56*	0.80	0.24
∑DDT	2.38±0.69		0.48±0.075		−4.89±0.69*	0.83	0.29
∑PCB	1.78±0.75		0.55±0.083		−5.40±0.75*	0.81	0.33
Lake whitefish ($n = 43$)[b]							
∑CHL	0.20±0.27	$-1.6 \times 10^{-3} \pm 3.3 \times 10^{-4}$		1.4±0.37	−0.46±0.27	0.66	0.34
CHB	0.18±0.043	$-7.5 \times 10^{-4} \pm 2.1 \times 10^{-4}$	0.20±0.053		−0.21±0.41	0.65	0.27
∑DDT	0.13±0.25	$-1.9 \times 10^{-3} \pm 4.7 \times 10^{-4}$	0.15±0.078	1.8±0.58	−0.83±0.55	0.64	0.43
∑PCB	0.14±0.052	$-2.0 \times 10^{-3} \pm 4.2 \times 10^{-4}$		2.1±0.46	0.079±0.34	0.55	0.45
Longnose sucker ($n = 18$)[c]							
∑CHL	0.24±0.11	$6.9 \times 10^{-3} \pm 2.5 \times 10^{-3}$			−3.0±1.1	0.49	0.22
CHB	0.29±0.087	$8.7 \times 10^{-3} \pm 2.0 \times 10^{-3}$	0.16±0.054		−4.1±0.96*	0.75	0.18

Note: Intercepts with an asterisk were significantly different from 0 at $P < 0.05$.

[a]The log-transformed weight and lipid were used in regressions.

[b]The log-transformed age was used in regressions.

[c]Length was used instead of weight.

$$\log \sum CHL = 0.16(\pm 0.051)\delta^{15}N - 0.85(\pm 0.53), \quad r^2 = 0.22,\ SE_{est} = 0.49$$

$$\log \sum DDT = 0.22(\pm 0.045)\delta^{15}N - 0.92(\pm 0.47), \quad r^2 = 0.41,\ SE_{est} = 0.43$$

$$\log \sum PCB = 0.24(\pm 0.057)\delta^{15}N - 0.99(\pm 0.59), \quad r^2 = 0.33,\ SE_{est} = 0.54$$

$$\log CHB = 0.23(\pm 0.059)\delta^{15}N - 0.74(\pm 0.62), \quad r^2 = 0.29,\ SE_{est} = 0.57$$

whereas lipid-adjusted ∑HCH or ∑CBZ concentrations were not related to lake trout $\delta^{15}N$ ($P = 0.55$ and 0.53, respectively; Fig. 4).

Size and age of the fishes were not significant predictors of organochlorine concentrations within populations. However, some significant relations existed for lake trout, lake whitefish, and longnose suckers when all data were pooled. Length or weight (but not age) explained between 26 and 38% of the variability in organochlorine concentrations in lake trout (relations not shown). Significant negative relations ($r^2 < 0.23$) were observed between organochlorine concentrations and length or weight of lake whitefish and were the result of analysing larger less-contaminated fish from Fox Lake. Concentrations of ∑CHL, ∑PCB, ∑DDT, and CHB were also significantly related to length of longnose suckers and explained more of the variability in organochlorine concentrations than lipid for ∑CHL and CHB.

Multiple regression analyses indicated that a combination of lipid, $\delta^{15}N$, and weight or age explained between 69 and 90% of the variability in organochlorine concentrations in lake trout and burbot tissues (Table 4). ∑CHL, ∑DDT, and ∑PCB concentrations in northern pike were significantly related to lipid and $\delta^{15}N$ ($r^2 = 0.83$–0.81), but no combination of variables improved upon results of linear regression analyses for ∑HCH, ∑CBZ, and CHB in this species. Concentrations of ∑CHL, ∑DDT, ∑PCB, and CHB in lake whitefish were significantly related to lipid, weight, age (except CHB), and $\delta^{15}N$ (CHB and ∑DDT only), and these variables explained more variability (55–66%) than any simple linear regression analysis (Table 3). In addition to lipid, ∑CHL and CHB concentrations were significantly related to weight and $\delta^{15}N$ (CHB only) in longnose sucker. However, for most other contaminants in longnose sucker and round whitefish, lipid was the best predictor of organochlorine concentrations (see Table 3).

Discussion

Our ability to understand the importance of trophic positioning

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in organochlorine bioaccumulation in fishes has, until recently, been limited by difficulties in determining the long-term dietary habits of individuals. In previous studies, all lake trout within a population were presumed to have comparable dietary habits, and the effect of trophic positioning on PCB and ∑DDT bioaccumulation was examined across systems differing in underlying food chain length (class 1–3) using integer-based trophic level (TL) assignments (Rasmussen et al. 1990; Bentzen et al. 1996). While much of the variability in organochlorine concentrations in lake trout populations could be ascribed to among-lake differences in trophic positioning, it was not possible to quantify the effects of increasing food chain length on organochlorine bioaccumulation using these discrete groupings. Vander Zanden and Rasmussen (1996) used mean diet data for lake trout and forage fishes from several lakes within each class to calculate an actual trophic position of these populations (lake trout from class 1, at mean TL of 3.55; class 2, at TL 3.89; class 3, at TL 4.38). They observed that this continuous-measure trophic position was a better predictor of PCB concentrations in lake trout ($r^2 = 0.83$) than the discrete class assignments used previously ($r^2 = 0.72$), because it took into account omnivorous feeding behaviour within both the forage fish and lake trout populations. While stomach content analyses provide short-term information on the dietary habits of fishes, the incorporation of $\delta^{15}N$ measurements into bioaccumulation studies removes the uncertainty associated with diet-based trophic assignments (Vander Zanden and Rasmussen 1996) and represents an organism's longer term trophic positioning by measuring what is assimilated and incorporated into an organism from its diet over the time period of tissue turnover, likely months to years in slow-growing populations (Hesslein et al. 1993).

The top predators from the three subarctic lakes we studied occupied significantly different trophic positions as determined by $\delta^{15}N$ measurements, and these differences were not due to variability in $\delta^{15}N$ at the base of these food webs (Fig. 1), as was observed in a study by Cabana and Rasmussen (1996) for lakes impacted by anthropogenic inputs of nitrogen. Across lakes, concentrations of most organochlorines in lake trout, burbot, and northern pike from these subarctic lakes were significantly related to their $\delta^{15}N$, demonstrating that top predators from lakes with longer food chains have higher concentrations of organochlorines. The effect of food-chain length on organochlorine bioaccumulation varied with the lipophilicity of the compounds, with the more lipophilic organochlorines having higher log organochlorine – $\delta^{15}N$ slopes and r^2 than the less lipophilic organochlorine groups (Table 3, Fig. 3; ∑DDT and CHB > ∑HCH in lake trout; ∑DDT, ∑PCB, and CHB > ∑HCH and ∑CBZ in burbot; ∑DDT, ∑PCB, and CHB > ∑HCH in northern pike). These differences in slopes can be attributed to the kinetics of these organochlorines in fishes; the more lipophilic organochlorines have longer elimination half-lives in biota than the less lipophilic compounds (de Boer et al. 1994; Sijm et al. 1992) and, therefore, accumulate to higher concentrations in an organism's tissues (Oliver and Niimi 1988) and bioaccumulate to a greater extent through the food chain (Thomann 1989). Similar organochlorine–$\delta^{15}N$ relations have also been observed through the food web; the slopes and coefficients of determination for ∑DDT or CHB versus $\delta^{15}N$ were greater than that found for ∑HCH versus $\delta^{15}N$ in biota from Lake Laberge (Kidd et al. 1995*b*). Although the relations between organochlorines and $\delta^{15}N$ would be best examined using individual congeners with known K_{ow}s, the differences in log organochlorine – $\delta^{15}N$ slopes observed herein suggest that trophic positioning was of greater significance in the bioaccumulation of the more than less lipophilic contaminants.

Lipid content increases with the trophic position of organisms (Rasmussen et al. 1990; Oliver and Niimi 1988) making it difficult to delineate the effects of these factors on organochlorine bioaccumulation in fishes. Rasmussen et al. (1990) speculated that, because the concentrations of PCBs in lake trout increased at a proportionally greater rate with increasing food-chain length than did lipid, trophic positioning was a more important factor in organochlorine bioaccumulation in this species. The confounding effects of lipid on organochlorine concentrations may be removed using ANCOVA (Hebert and Keenleyside 1995), and the resultant adjusted contaminant values then examined for the effects of trophic positioning. Bentzen et al. (1996) recently demonstrated that after accounting for the variation in lipid content using ANCOVA, a significant among-class difference in lake trout PCB and ∑DDT concentrations remained, which they attributed to differences in underlying food chain length and (or) inputs of contaminants. However, they were unable to quantify the effects of trophic positioning, because they used a categorical variable to describe food chain length. We also found that after removing the effects of lipid from the lake trout organochlorine data, significant among-lake differences in organochlorine concentrations remained. Lipid-adjusted concentrations of the more lipophilic organochlorines, ∑CHL, ∑DDT, ∑PCB, and CHB, in lake trout muscle were significantly related to their $\delta^{15}N$, demonstrating that, for these contaminants but not for the less lipophilic ∑HCH and ∑CBZ, trophic positioning is a significant factor in organochlorine bioaccumulation over and above lipid (Fig. 4).

Lake trout and whitefish muscle and burbot liver were analysed for organochlorines in this study because of concerns about human consumption of contaminated fishes from these lakes. Significant differences between muscle and whole-body concentrations of organochlorines occur for salmonid species (Niimi and Oliver 1983, 1989). In laboratory experiments, Niimi and Oliver (1983) found that whole rainbow trout (*Oncorhynchus mykiss*) had up to 10 times more lipid and 2–4 times more PCB than muscle tissue. Similar differences were observed in field studies of whole body and muscle analyses of coho salmon (*Oncorhynchus kisutch*) and several species of trout from Lake Ontario. For all of these species, Niimi and Oliver (1989) found whole fishes had considerably higher concentrations of PCB, DDT, HCH, CBZ, and CHL congeners (two- to five-fold) than muscle tissues, and this corresponded to differences in tissue lipid content of a similar magnitude. The internal distribution of lipids varies considerably among species and may depend upon the reproductive strategy (Larsson et al. 1996) and status (Larsson et al. 1993) of the fishes. Burbot from Lake Laberge have low muscle lipid content (0.3%) and concentrations of ∑DDT and CHB that are up to 700 times lower than those found in their fatty (up to 50%) liver tissues (J. Eamer, Environment Canada, Whitehorse, Yukon, personal communication). Pike also have low lipid in their muscle tissues (this study; Larsson et al. 1993), and this lipid consists mainly of phospholipids, a class of lipids

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with a lower affinity for organochlorines than the storage fats, triglycerides (Ewald and Larsson 1994). Whole-body analyses may facilitate interspecific comparisons of organochlorine burdens and interpretations of the relative importance of lipid and trophic positioning on contaminant bioaccumulation in these fishes.

Lake Laberge has previously supported commercial, sport, and subsistence fisheries and, possibly as a result of heavy fishing pressure, has anomalously low biomasses of lake trout and lake whitefish and a high biomass of burbot when compared with other regional lakes (de Graff and Mychasiw 1994). Populations of fish from exploited lakes grow faster than those from non-exploited lakes (e.g., Healey 1978), probably because of reduced intraspecific competition and an increased forage base. Although faster growth has been associated with decreased contaminant concentrations within a species complex (Hammar et al. 1993), faster-growing lake trout will switch from a diet of invertebrates, sculpin (*Cottus* spp.), and yellow perch (*Perca flavescens*) to one consisting mainly of cisco (*Coregonus* spp.) at an earlier age than slower growing populations (Trippel and Beamish 1993) and, by doing so, may increase their tissue concentrations of organochlorines. In Lake Laberge, trout grow faster, have a higher average condition factor (weight/length ratio: Laberge, 1.26; Kusawa, 1.01; Fox, 1.10), and are mainly piscivorous when compared to trout from Kusawa and Fox lakes and other populations in lakes on the Yukon River system (Thompson 1996*a*, 1996*b*). Summer stomach content analyses revealed that 85% of all lake trout ($n = 84$) from Lake Laberge were strict piscivores, as compared with 38 and 15% of those fishes examined from Kusawa ($n = 64$) and Fox ($n = 40$) lakes, respectively. Burbot and northern pike from Laberge were also strict piscivores, unlike the populations from Fox Lake, which fed both upon fish and invertebrates. In addition, 5% of all lake whitefish from Laberge ($n = 220$) were strict piscivores, which was not observed in Kusawa or Fox lakes and would explain the higher $\delta^{15}N$ in Laberge's whitefish population (Kidd 1996). Lake trout and the other top predators in Laberge may have had higher concentrations of organochlorines when compared with the same species from other northern lakes, not only because they fed at a higher trophic position but because they began feeding at a higher trophic level, and thus on more contaminated prey, at a younger age.

Previous studies have found that size and (or) age were important predictors of the levels of contaminants in fishes, with contaminant concentrations increasing with one or more of these variables. Weight, length, or age are significant predictors of organochlorine concentrations in salmonids (Larsson et al. 1996; Rasmussen et al. 1990; Bentzen et al. 1996), and in burbot liver (Muir et al. 1990) from temperate and arctic lakes. Unlike these studies, we found that organochlorine concentrations were not related to age of any fish species and that age was only a significant variable when included in the multiple regression analyses for burbot and lake whitefish (Table 4). Similarly, weights or lengths alone were not significant predictors of organochlorine concentrations in burbot, northern pike, and round whitefish and were poor predictors of contaminant burdens in lake trout and lake whitefish as compared with the results of regression analyses for $\delta^{15}N$ or lipid. The only exception was found for longnose sucker; $\sum$CHL and CHB concentrations were better predicted by length than any other variable of interest.

Dated sediment cores and large-volume water samples were collected and analysed from all three of the lakes in this study to determine if Lake Laberge was contaminated by spills or leachates from dumpsites in the City of Whitehorse, located upstream from the lake. No evidence of point-source contamination of CHB was found for Laberge, because maximal and surface fluxes of this contaminant were similar between sites (Kidd et al. 1995*a*). Although results of sediment core analyses showed elevated historical inputs of $\sum$PCBs and $\sum$DDT to Laberge prior to 1970, which are most likely the result of past industrial activity and mosquito and black fly control, respectively (DIAND 1993), concentrations after this period are comparable with those found in cores from Fox and Kusawa lakes. Concentrations of $\sum$HCH, $\sum$CBZ, and $\sum$CHL in the sediment cores were maximal in the 1970s and comparable among lakes (D. Muir, Department of Fisheries and Oceans, Winnipeg, MB R3T 2N6, unpublished data). Concentrations of $\sum$CBZ, $\sum$HCH, $\sum$CHL, CHB, and $\sum$PCB in water from Laberge were similar to or lower than levels found in Fox and Kusawa lakes, and $\sum$DDT in water samples was low (0.1 ng/L) but slightly higher in Laberge than was found in Fox, Kusawa, and other sites on the Yukon River system (Alaee and Gregor 1994). Organochlorine analyses of invertebrate samples (zooplankton, chironomids, mollusks, and trichopterans) from Laberge, Kusawa, and Fox lakes did not reveal any consistent differences among lakes; only chironomid samples from Laberge had significantly higher $\sum$CBZ and $\sum$DDT when compared with the same taxa from either Fox or Kusawa lakes (Kidd 1996). Generally, no evidence of high contemporary inputs of organochlorines to Laberge was found, indicating that the among-lake differences in fish concentrations were more likely due to food chain length than variability in the inputs of contaminants to these systems.

In conclusion, lipid content and (or) trophic positioning, as determined by $\delta^{15}N$, were significant predictors of the contaminant concentrations in the top predators lake trout, burbot, and northern pike from these subarctic lakes in Yukon Territory. Although $\delta^{15}N$ was a better individual predictor (higher r^2) of the more lipophilic than the less lipophilic contaminants, and lipid was a better predictor of the less lipophilic than the more lipophilic contaminants, a combination of the two factors explained more of the variability in the lake trout organochlorine data than either alone. After lipid adjusting the organochlorine data using ANCOVA, $\delta^{15}N$ remained a significant predictor of the concentrations of all contaminants in lake trout but $\sum$HCH and $\sum$CBZ, demonstrating that trophic positioning was an important factor in the bioaccumulation of the more lipophilic organochlorines such as $\sum$DDT and $\sum$PCB. The use of biotic $\delta^{15}N$ measurements in contaminant studies provides a method for quantifying the effects of underlying food web structure and for delineating the effects of lipid and trophic level on organochlorine bioaccumulation within species.

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Impacts of density-dependent growth and maturation on assessment advice to rebuild depleted U.S. silver hake (*Merluccius bilinearis*) stocks

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Abstract: We examined how density-dependent growth and maturation rates would affect scientific advice to rebuild U.S. silver hake (*Merluccius bilinearis*) stocks to a minimum biomass threshold (SSB_{50}) using an age-structured stochastic projection model. Uncertainty in recruitment and initial stock sizes at age was incorporated to more realistically capture the stochastic nature of the stock's dynamics through time. Performance measures including spawning stock biomass (SSB_t), risk ($prob(SSB_t < SSB_{50})$), and fishery yields were examined under model assumptions of static life history parameters in contrast with density-dependent parameters for various constant fishing mortality rate (F) harvest policies. Model results indicated that unless compensatory mechanisms are accounted for, scientific advice may be too optimistic and lead to unrealistic expectations of F targets to achieve stock rebuilding, requiring longer rebuilding times and entailing more risk and unrealized sustainable yield. For instance, projected median southern stock SSB_t exceeded the SSB_{50} of 104 000 metric tons (t) with 40% risk to the stock by year 20 from harvesting at $F = 0.50$. However, under the density-dependent assumption, median SSB_t did not rebuild as expected, and after 20 years reached only 75 000 t with 85% risk to the stock. Also under this assumption, median yields increased from 9000 to 27 000 t by year 20 but were 30–40% lower than what would have been expected under density independence.

Résumé : Nous avons cherché à voir si l'hypothèse d'une croissance et d'une maturation dépendant de la densité de population peut influer sur les avis scientifiques concernant le rétablissement des stocks américains de merlu argenté (*Merluccius bilinearis*) au seuil de biomasse minimum (BSR_{50}); pour ce faire, nous nous sommes servi d'un modèle de projection stochastique structuré en fonction de l'âge. Nous avons introduit un facteur d'incertitude, applicable au recrutement et à l'effectif initial des stocks selon l'âge, pour reproduire de façon plus réaliste la nature stochastique de la dynamique des stocks. Comme mesures de performance, nous avons notamment utilisé la biomasse du stock reproducteur (BSR_t), le risque ($prob(BSR_t < BSR_{50})$) et la production de la pêche en posant, comme caractéristiques d'application du modèle, des paramètres de cycle biologique statiques par opposition aux paramètres dépendant de la densité qui caractérisent les politiques d'exploitation basées sur des valeurs constantes de la mortalité par pêche (F). D'après les résultats que nous avons obtenus avec ce modèle, à moins que des mécanismes de compensation soient mis en évidence, les avis scientifiques pourraient être trop optimistes et donner lieu à des visées irréalistes en ce qui touche les valeurs de F permettant de rétablir les stocks, le rétablissement étant en fait moins rapide et comportant plus de risques, et la produciton durable ne pouvant se concrétiser. Par exemple, la BSR_t moyenne prévue pour le stock du sud dépassait le BSR_{50} de 104 000 tonnes métriques (t), et le stock était exposé à un risque s'élevant à 40% à la vingtième année avec un taux d'exploitation à $F = 0{,}50$. Toutefois, en supposant la relation de dépendance avec la densité, la BSR_t moyenne ne se rétablissait pas comme prévu et n'atteignait que 75 000 t au bout de 20 ans, alors que le risque pour le stock était évalué à 85%. En outre, suivant cette hypothèse, les rendements moyens augmentaient de 9 000 à 27 000 t en 20 ans, mais étaient de 30 à 40% inférieurs à ce qu'on aurait prévu en supposant l'absence de relation de dépendance avec la densité.
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Introduction

Silver hake (*Merluccius bilinearis*) is a widely distributed gadoid whose range extends from Newfoundland to South Carolina (Bigelow and Schroeder 1953), but is most abundant between Nova Scotia and the New York Bight. In U.S. waters, silver hake is one of the most abundant demersal species and plays an important role, both as a predator and prey, in regulating fish communities of the northeast continental shelf ecosystem (Edwards and Bowman 1979). The silver hake population in waters off the northeastern United States is composed of two stocks (Almeida 1987): a northern stock extending from the Gulf of Maine to the northern flank of Georges Bank and a

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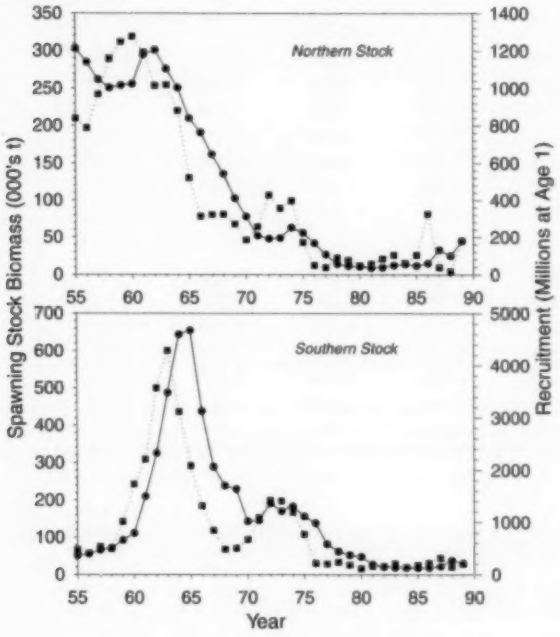

Fig. 1. Estimated SSB (solid lines) and recruitment to age 1 (broken lines) for U.S. northern and southern stocks of silver hake, 1955–1989.

southern stock extending from the southern portion of Georges Bank to the mid-Atlantic Bight.

Because of its availability and high abundance, silver hake has historically been an important commercial resource for both U.S. and distant water fleets. While total landings from both stocks peaked at over 350 000 metric tons (t) in the mid-1960s under intensive exploitation by distant water fleets (particularly the (previously) U.S.S.R.), landings declined rapidly thereafter to only 20 000 t in the early 1970s due to overfishing. Spawning stock biomass (SSB) of both stocks subsequently plummeted from the mid-1960s to the early 1970s, declining from 300 000 t to only 50 000 t in the northern stock and from 650 000 t to only 150 000 t in the southern stock (Helser et al. 1995; Fig. 1). Although both stocks recovered slightly during the mid-1970s, SSB and recruitment had declined again by the early 1980s. For the northern stock, SSB dropped from 65 000 to 14 000 t, while for the southern stock, SSB declined from 180 000 t to only about 20 000 t. Over the last decade, SSB and recruitment have remained at historic low levels (Helser and Mayo 1994).

Despite severe declines in population abundance, silver hake, like many *Merluccius* species worldwide, has shown a marked resilience in the face of heavy exploitation (Alheit and Pitcher 1995). Density-dependent growth and maturation rates may be a key factor in the resiliency of silver hake stocks where low population abundance is associated with rapid growth and early maturation. Several studies confirm that population density is an important mechanism in regulating the dynamics of both silver hake stocks in waters off the northeastern United States. These include (*i*) interspecific competition with Atlantic mackerel (*Scomber scombrus*) and potential predator–prey relationships with haddock (*Melanogrammus aeglefinus*) and Atlantic cod (*Gadus morhua*) (Sissenwine et al. 1982), (*ii*) cannibalism (Edwards and Bowman 1979), and (*iii*) intraspecific competition leading to density-dependent growth (Ross and Almeida 1986; Helser and Almeida 1997) and density-dependent age-specific sexual maturation (Helser and Almeida 1997).

When the goal of fishery management is to rebuild depleted fishery resources, such as the U.S. silver hake stocks, information on compensatory mechanisms needs to be incorporated into assessment models to develop realistic expectations from harvest policies. Age-structured stochastic projection models have become increasingly important for evaluating management policies for rebuilding depleted stocks. In particular, age-structured stochastic projection models are being used as part of the scientific advice for some stocks in the northeast region of the United States (Rosenberg and Restrepo 1994). The advantage of these projection models is that they can characterize the uncertainty and inherently stochastic nature of stock dynamics over time under various harvest policies. This is accomplished by incorporating uncertainty from process error associated with variable recruitment (Smith et al. 1993) and uncertainty from estimation error arising from estimates of initial stock sizes (Conser 1994). Variability in life history parameters such as growth, sexual maturity, and natural mortality can also lead to uncertainty in assessment advice (Smith et al. 1993; Rosenberg and Restrepo 1994). Few studies have explicitly incorporated density-dependent life history parameters directly into age-structured projection models (Overholtz et al. 1991), and our analysis represents the first of its kind in evaluating the impact of compensatory responses of life history parameters on rebuilding a depleted groundfish stock in the Northwest Atlantic.

In this study, we used a stochastic projection model to examine the impact of assumptions regarding compensatory changes in age-specific growth and sexual maturation on rebuilding scenarios for the depleted U.S. silver hake stocks. The management implications of assuming static life history parameters in contrast with density-dependent parameters were examined for several performance measures including SSB, risk to the stocks, and fishery yields. The stochastic projection model incorporated two random components: recruitment and initial population sizes. Recruitment was modeled as a Ricker stock–recruitment curve with lognormal error. Initial population sizes were randomly generated by adding a normal random variate to each element of the estimated population size at age vector. Moreover the model included an explicit description of density-dependent changes in age-specific growth and sexual maturity. The projection model was applied to simulate the responses of the depleted U.S. silver hake stocks to various harvest policies, and the simulation results illustrate the importance of considering the strength of compensatory responses for depleted stocks.

Materials and methods

We used an age-structured model of an iteroparous fish population where changes in population abundance due to recruitment, natural mortality, and fishing mortality served as the basis for projections (Brodziak and Rago, unpublished manuscript). Because the impact of population density on growth and maturation rates was found to be

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Table 1. Input parameter values used to initialize projection model simulations.

Parameter[a]	Age a 1	2	3	4	5	6	7+
Northern stock							
Selection at age (S)	0.281	0.500	1.000	1.000	1.000	1.000	1.000
Fraction mortality (p)	0.660	0.660	0.660	0.660	0.660	0.660	0.660
Catch weight (W_C)	0.078	0.144	0.218	0.301	0.394	0.559	0.674
Stock weight (W_S)	0.017	0.076	0.177	0.312	0.466	0.632	0.674
Natural mortality (M)	0.400	0.400	0.400	0.400	0.400	0.400	0.400
Fraction mature (V)	0.080	0.700	0.990	1.000	1.000	1.000	1.000
Stock size in millions (N)[b]	16.9	25.6	134.8	10.9	3.2	0.5	0.0
Southern stock							
Selection at age (S)	0.100	0.500	1.000	1.000	1.000	1.000	1.000
Fraction mortality (p)	0.500	0.500	0.500	0.500	0.500	0.500	0.500
Catch weight (W_C)	0.075	0.122	0.178	0.238	0.322	0.425	0.522
Stock weight (W_S)	0.012	0.091	0.216	0.348	0.442	0.522	0.551
Natural mortality (M)	0.400	0.400	0.400	0.400	0.400	0.400	0.400
Fraction mature (V)	0.090	0.800	0.99	1.000	1.000	1.000	1.000
Stock size in millions (N)[b]	147.2	209.1	72.3	20.9	1.8	0.0	0.1

[a]Input parameters taken from Helser and Mayo (1994).
[b]Starting stock sizes taken from NEFSC (1990).

substantial for both silver hake stocks in U.S. waters (Helser and Almeida 1997), we modified the model to account for density-dependent changes in mean weight at age and fraction sexually mature at age.

Age-structured projection model

For each hake stock, population abundance was represented as the vector $\mathbf{N}(t)$:

$$(1) \qquad \mathbf{N}(t)=\begin{bmatrix} N_1(t) \\ N_2(t) \\ \cdots \\ \cdots \\ N_7(t) \end{bmatrix}$$

where $N_a(t)$ was the number of age a fish alive at the beginning of the year. Here, we used a plus-group, $N_7(t)$, to accumulate the number of fish that were 7 years old or older. Population survival at age from year $t-1$ to year t was calculated in the standard manner for instantaneous fishing and mortality rates. To describe annual survival through mortality, let M_a denote the constant instantaneous natural mortality rate and let $F_a(t)$ denote the instantaneous fishing mortality rate for age a fish in year t of the time horizon. For both hake stocks the natural mortality rate was taken to be $M = 0.4$ (NEFSC 1990). Then, population size at age in year t is given by

$$(2) \qquad N_a(t) = N_{a-1}(t-1)\cdot e^{-M_{a-1}-F_{a-1}(t-1)} \text{ for } a = 1 \text{ to } 6$$

$$N_7(t) = N_7(t-1)\cdot e^{-M_7-F_7(t-1)} + N_6(t)(t-1)\cdot e^{-M_6-F_6(t-1)}.$$

Note that survival for the plus-group involves an age 7+ and an age 6 component.

Population sizes at age serve as the starting conditions for the projection analyses; we used starting values (Table 1) estimated with a tuned-VPA model (NEFSC 1990; Conser 1994). Because estimates of population abundance typically are estimated with error, we chose to randomly generate a set of 500 initial population vectors, $\{\mathbf{N}^{(1)}(1), \mathbf{N}^{(2)}(1),..., \mathbf{N}^{(500)}(1)\}$, to represent the sampling distribution of the estimator of $\mathbf{N}(1)$. For each element of the initial population vector ($t = 1$), we generated an independent normal error term using a coefficient of variation (CV) of 25%.

The numbers of new recruits that enter the fishery at age 1 (1 year after hatching) at the beginning of each year are also determined through a stochastic process that is dependent on spawning biomass. The SSB in year $t-1$ that produced recruits in year t is

$$(3) \qquad \text{SSB}(t) = \sum_{a=1}^{7} W_{S,a}V_aN_a(t)e^{-p[M_a+F_a(t)]}$$

where SSB(t) is the sum over all age-classes of the weight of mature fish at the midpoint of the spawning season, V_a is the fraction of age a fish that are mature, $W_{S,a}$ is the average weight of an age a fish, and p is the average fraction of total annual mortality that occurs from January 1 to the midpoint of the spawning season for the fish stock.

We used a Ricker (1954) curve with lognormal error to generate random recruitment from SSB during the previous year. We evaluated the Beverton–Holt (1957) and Shepherd (1982) stock–recruitment models but found that the Ricker curve provided the best fit to the data for both stocks. This curve provides a parametric model for simulating recruitment where survival to recruitment age is density dependent and subject to stochastic variation. The Ricker curve with lognormal error generates recruitment as

$$(4) \qquad N_1(t) = a\cdot\text{SSB}(t-1)\cdot e^{-b\cdot\text{SSB}(t-1)}\cdot e^{w}$$

where $w \sim N(0, \sigma_w^2)$; the stock–recruitment parameters a, b, and σ_w^2 were estimated with nonlinear regression of the stock–recruitment data (Table 2). We did not explicitly consider serial autocorrelation in fitting the stock–recruitment data or correct for it in predictions, although evidence for it existed (i.e., Durbin–Watson test statistic = 0.86, $p < 0.05$). Positive autocorrelation in time series data produces nominal variances about parameter estimates that are smaller than they should be (Bence 1995). Its impact on the simulation results would lead to higher variability around yield and biomass trajectories than that reported.

The catch of age a fish in year t in the fishery is given by the standard catch equation, denoted as $C_a(t)$:

$$(5) \qquad C_a(t) = \frac{F_a(t)}{M_a + F_a(t)}\left|1 - e^{-[M_a+F_a(t)]}\right| N_a(t).$$

Then, the total landed weight in year t, denoted by $L(t)$, is

$$(6) \qquad L(t) = \sum_{a=1}^{A} C_a(t)W_{C,a}.$$

Population harvest is determined by setting the annual fishing

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Table 2. Estimated coefficients used to parameterize density-dependent relationships for growth, sexual maturity, and stock–recruitment.

Functional relationship	Age a	Parameter coefficient (SE) α	β_1	β_2	β_3	σ_w^2
Northern stock						
Weight at age (W_C)	2	5.301 (0.039)	–0.0011 (0.0001)	—	—	—
	3	5.639 (0.047)	–0.0012 (0.0002)	—	—	—
	4	5.905 (0.087)	–0.0012 (0.0004)	—	—	—
Fraction mature (V)		–4.296 (0.359)	2.7451 (0.1680)	–0.0024 (0.0008)	–0.0009 (0.0003)	—
Stock–recruitment (N_1)		5.057 (1.025)	0.0021 (0.0010)	—	—	0.602
Southern stock						
Weight at age (W_C)	2	5.360 (0.081)	–0.0009 (0.0001)	—	—	—
	3	5.664 (0.055)	–0.0008 (0.0001)	—	—	—
	4	5.939 (0.093)	–0.0007 (0.0002)	—	—	—
Fraction mature (V)		–5.228 (0.272)	3.7050 (0.1421)	–0.0004 (0.0001)	–0.0008 (0.0001)	—
Stock–recruitment (N_1)		9.442 (1.644)	0.0022 (0.0007)	—	—	0.591

mortality rate at age, $F_a(t)$. In this case, the age-specific fishing mortality rate for age a fish is the product of the fully recruited fishing mortality rate, denoted by $F(t)$, and the partial recruitment to $F(t)$ for age a fish (age-specific selectivity), denoted by $S_a(t)$. That is:

$$F_a(t) = F(t)S_a. \quad (7)$$

Under the assumption of density independence the life history parameters, V_a and $W_{S,a}$, used to calculate spawning biomass and landings in the above equations are static and remain constant (initial values given in Table 1) under any population size. In contrast, the density-dependent assumption allows for the life history parameters to vary in response to changing population size. Density-dependent growth of silver hake was modeled as negative linear functions of stock abundance. Natural logarithms of mean weights at ages 2 through 4 in the landings were regressed on SSB between 1962 and 1980. These years were chosen for analysis because they encompassed a dynamic range of population biomass (Fig. 1). Regression results were statistically significant ($p < 0.01$) for both northern and southern silver hake (Table 2). These were of the form

$$\ln(W_{C,a}) = \alpha_a - \beta_a \cdot \mathrm{SSB} \quad (8)$$

where $W_{C,a}$ is mean weight at age a, SSB is average spawning stock biomass (thousands of metric tons) over the number of years prior to time t (i.e., $t = a - 1$), and α_a and β_a are regression coefficients. Regression coefficients are given in Table 2 and regression results are illustrated in Fig. 2. The functional relationship between mean weights at age of landings and stock abundance were also applied to changes between stock weights at age ($W_{S,a}$) and abundance.

The proportion of sexually mature fish was modeled as a response surface to both age and population abundance in which an age, an abundance, and an age–abundance interaction parameter were estimated (Helser and Almeida 1997) using logistic regression. The predicted proportion mature at age was predicted from

$$V_a = \frac{e^{(\alpha+\beta_1 A+\beta_2 N+\beta_3 A N)}}{1 + e^{(\alpha+\beta_1 A+\beta_2 N+\beta_3 A N)}} \quad (9)$$

where V_a is the proportion of fish mature, A is age, N is population abundance (millions of fish) summed over all ages, and α, β_1, β_2, and β_3 are regression coefficients. Results of the logistic regression along with coefficients are given in Table 2 and are illustrated in Fig. 3.

Risk and SSB thresholds

In our analysis, we defined "risk" as the probability of the SSB falling below a threshold. This definition implies that a there is some threshold level of SSB below which there is concern or risk that the stock will be unable to generate sufficient recruitment for rebuilding. In general, biomass thresholds are commonly used biological reference points with a biological basis for their calculation, generally from empirical stock and recruitment data. We estimated a recruitment overfishing threshold for SSB from the fitted Ricker stock–recruitment function and defined it as the level of SSB corresponding to one-half theoretical maximum recruitment (Meyers et al. 1994) and denoted it as SSB_{50}. For the southern stock, SSB_{50} was 104 000 t, while it was 110 000 t for the northern stock. Within the context of risk, we used these estimates of SSB_{50} for evaluating whether stock rebuilding targets would likely be met from a particular harvesting policy. The number of times that SSB met or exceeded the SSB_{50}, denoted by K_{50}, was computed for each year using the entire set of simulations (stochasticity from recruitment and initial stock sizes). Given this, the risk to the stock was defined as the probability that SSB_{50} would not be met in year t, and risk was computed as

$$\mathrm{prob}(\mathrm{SSB}_t < \mathrm{SSB}_{50}) = 1 - \frac{K_{50}}{K} \quad (10)$$

where K was the total number of simulation runs.

To provide a baseline from which to contrast the impact of density-dependent life history parameters on management advice, we first conducted the simulation analysis with a set of constant fishing mortality rates (F) under the assumption of density independence. Here, F ranged from 0.1 to 1.0 by 0.1 increments. For these simulation runs, we used a goal of rebuilding the depleted U.S. silver hake stocks to the SSB threshold within 20 years and defined risk based on the probability that the SSB threshold was not met. In particular, a harvest policy was considered generally unacceptable if the risk (probability that SSB_{50} was not met) was greater than 0.5. That is, a harvest policy put the stock at unacceptable risk in year t of the time horizon if $\mathrm{prob}(\mathrm{SSB}_t < \mathrm{SSB}_{50}) > 0.5$. The objectives of these analyses were twofold: (*i*) to evaluate which constant F would achieve the rebuilding target for each stock under the assumption of density independence and (*ii*) given a constant F harvest policy that would rebuild the stocks, to evaluate the risk that would result from assuming that growth and maturation rates were density independent (static parameters) when in fact these parameters were density dependent.

Results

Constant F harvest policies: density independence

Rebuilding depleted U.S. silver hake stocks to SSB thresholds was possible within 20 years, but the time horizon and constant F harvest rates required to achieve rebuilding varied widely between the two stocks. In the northern stock, median SSB

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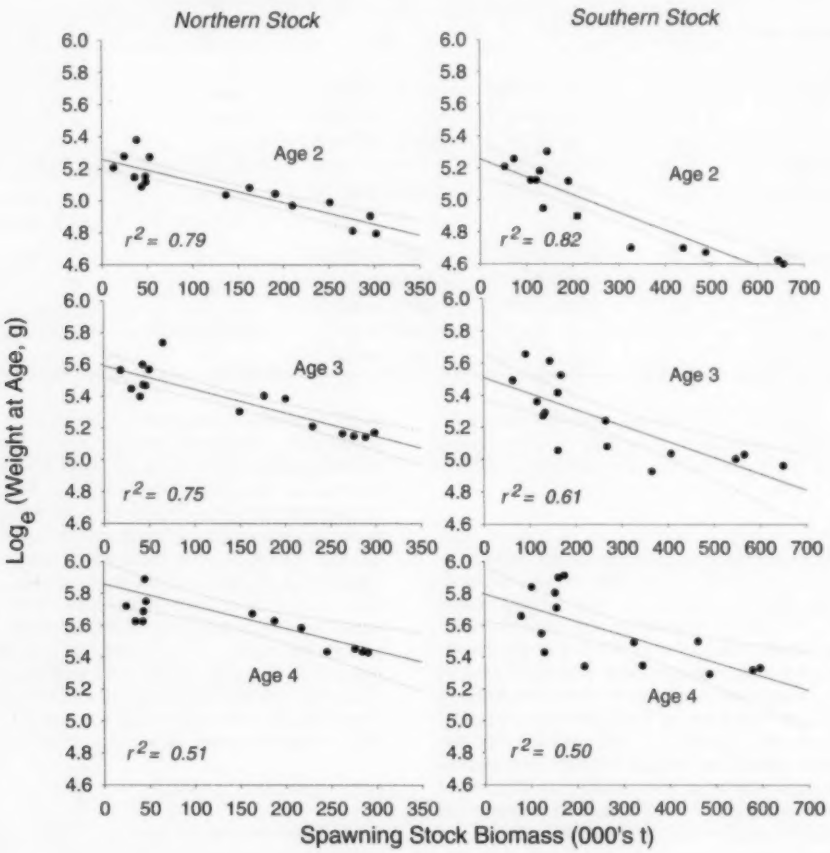
Fig. 2. Regressions of $\log_e$ (mean weight at age) for ages 2–4 on SSB for northern and southern stocks of silver hake. Points are observed mean weights at age, solid lines are predicted values from the regression, and broken lines are 95% confidence intervals about the regression line.



trajectories increased under constant F harvest rate policies for $F < 0.3$ (Fig. 4A). Fishing mortality rates greater than 0.3 did not lead to any stock rebuilding, and therefore, it was not necessary to examine biomass trajectories at greater harvest rates. Harvesting at $F = 0.1$ increased median SSB which reached the minimum biomass threshold of 110 000 t in the 15th year, while at $F = 0.2$, median SSB rebuilt more slowly, achieving the SSB threshold in year 20 (Fig. 4A). In the southern stock, median SSB trajectories increased for $F < 0.5$ and achieved the SSB threshold of 104 000 t within the 20th year (Fig. 4B). Constant F harvest rates of $F < 0.3$ resulted in rapidly increasing median SSB, but trajectories reached an asymptotic biomass level above the SSB threshold within the 10th year of the simulation. However, under $F = 0.5$ harvest policy, median SSB only gradually increased and achieved the threshold in the 18th year.

Because current SSBs for the stocks were well below the calculated SSB threshold, all harvest policies entailed considerable risk during the early years of the simulation, but risk decreased in later years (Figs. 4C and 4D). While risk to the northern stock declined gradually under a harvest policy of $F = 0.2$ to 60% after 20 years, only at $F = 0.1$ did risk reach acceptable levels (i.e., <50%). Here, risk to the stock declined to 50% in the 14th year of the simulation and reached 19% in year 20. In contrast, risk to the southern silver hake stock decreased more rapidly when $F < 0.3$ (Fig. 4D). Harvest policies of $F = 0.1$ and $F = 0.3$ entailed acceptable risk (50%) to the southern stock by the fifth and eighth year, respectively, and declined to <5% by year 15. While declining less rapidly, acceptable risk to the stock was achieved from harvesting at $F = 0.5$; risk fell below 50% by the 16th year and achieved 35% in the 20th year. On the basis of F examined under the density-independent assumption, $F = 0.1$ and $F = 0.5$ in the northern and southern stocks, respectively, were adopted as the minimally appropriate stock rebuilding guidelines to meet the stated management goals.

Expected median yield under the $F = 0.1$ and $F = 0.5$ policies was lower during the early years than from more conservative policies, but short-term losses were compensated for by significantly greater gains in yield in the later years of the simulation (Figs. 4E and 4F). For the northern stock, harvest policies of $F < 0.2$ gave initially lower yields of less than 5000 t in the early years (Fig. 4E). However, yields increased rapidly in later years, reaching 15 000 t by the 20th year. Similarly, for the southern stock, the most risk-averse harvesting policy ($F < 0.3$) did not translate into greatest benefit to the

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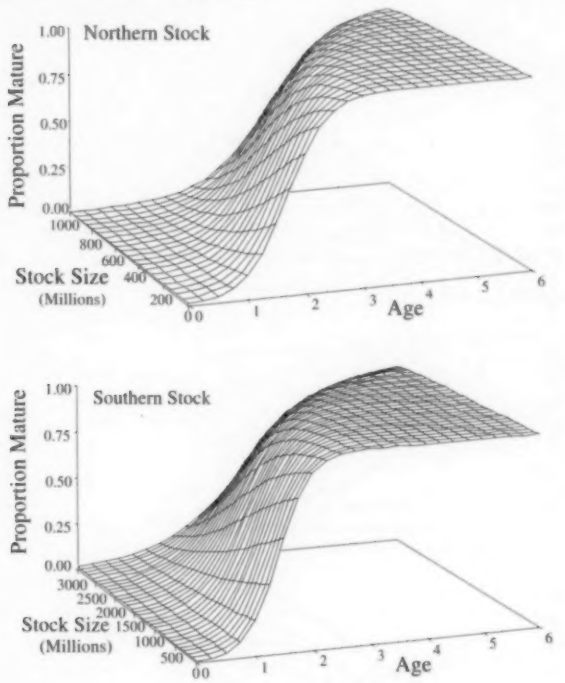
Fig. 3. Response surfaces of fraction mature as functions of age and population abundance for northern and southern silver hake stocks predicted by logistic regression.



fishery in terms of yield. Yield increased asymptotically to about 40 000 t from harvesting at $F = 0.1$, while at $F = 0.3$, yield increased more rapidly to about 70 000 t by the 20th year (Fig. 4F).

Constant *F* harvest policies: density dependence versus density independence

The management goals and benefits of harvesting at the chosen constant F of 0.1 and 0.5 for the northern and southern stocks, respectively, were not realized under density-dependent assumptions. Median SSB in the northern stock, while tracking closely under both assumptions, did not increase as expected under the density-dependent assumption in later years (>10), reaching only 60 000 t compared with 180 000 t under the density-independent assumption (Fig. 5A). Likewise in the southern stock, realized median SSB only gradually increased to about 75 000 t by the 20th year under density dependence, falling far short of the 130 000 t expected from the density-independent assumption. It is important to note that harvesting at $F = 0.1$ in the northern stock or $F = 0.5$ in the southern stock did not achieved their rebuilding targets within 20 years under the density-dependent assumption (Fig. 5B).

The risks to the stocks from harvesting at $F = 0.1$ and $F = 0.5$ for the northern and southern stocks, respectively, were more substantial than the risks generated under density independence. Over the 20-year simulation horizon, risk from harvesting at $F = 0.1$ under the density-dependent assumption never decreased below 90% for the northern stock compared with the 20% risk estimated under density independence (Fig. 5C). In addition, under the density-dependent assumption and constant harvest rate policy of $F = 0.5$ for the southern stock, risk also remained high (>80%) over the 20 years of the simulation compared with only 40% risk under density independence (Fig. 5D). Although the simulation time horizon was carried out only to 20 years, it appears likely that harvesting at $F = 0.1$ and $F = 0.5$ for the northern and southern silver hake stocks, respectively, would require many additional years to achieve the stated management goals.

Median yield to the fishery under density dependence was as much as 30% less than yield expected from simulation results under the density-independent assumption. Median yields under density dependence increased to only about 6000 t compared with 9000 t expected in the northern stock under the density-independent assumption (Fig. 5E). Similarly, in the southern stock, median yield increased to 27 000 t in contrast with 40 000 t under the density-independent assumption (Fig. 5F).

Long-term SSB, risk, and fishery yield: comparison of assumptions

To develop a longer term perspective from a broader array of constant F harvest policies and to contrast model results under the two different assumptions, simulations were conducted and the average of the median SSB, risk, and yield trajectories computed. The average of the 20-year series of median SSB, risk, and yield realizations would allow sufficient time for the stock to reach average long-term values. Average SSB from the simulations under the both assumptions declined exponentially as F increased from 0.1 to 1.0 by 0.1 increments (Figs. 6A and 6B). Under the density-dependent assumption, average SSB's from both stocks were considerably less than those expected under density independence; however, the differences between them diminished as fishing mortality increased.

Average risk under both assumptions in the northern stock was relatively high (>70%) even at lower constant F generally due to the slow rate over which SSB in the northern stock rebuilt. Average long-term risk experienced by the northern stock over the 20-year simulation under the density-dependent assumption was nevertheless greater than risk expected under density independence, even at the lowest F (<0.3) (Fig. 6C). Similarly, average risk in the southern stock under the density-dependent assumption was also higher than risk expected under density independence, but in both cases, risks to the stock were considerably less (<50%) under lower constant F (Fig. 6D).

For both stocks, average yield under either assumption increased to a maximum and then decreased as F increased, with maximum average yields occurring at $F = 0.2$ and $F = 0.3$ in the northern and southern stocks, respectively (Figs. 6E and 6F). Average yield under density dependence was markedly lower than yield generated under the density-independent assumption from the simulation over a wide range of different F. In particular, average yields from the stocks at their maximum under the density-dependent assumption were as much as 30% less than yields expected from density independence. For instance, harvesting at $F = 0.3$ (maximum) in the southern stock resulted in average yields of 23 000 and 33 000 t from density-dependent and density-independent assumptions, respectively.

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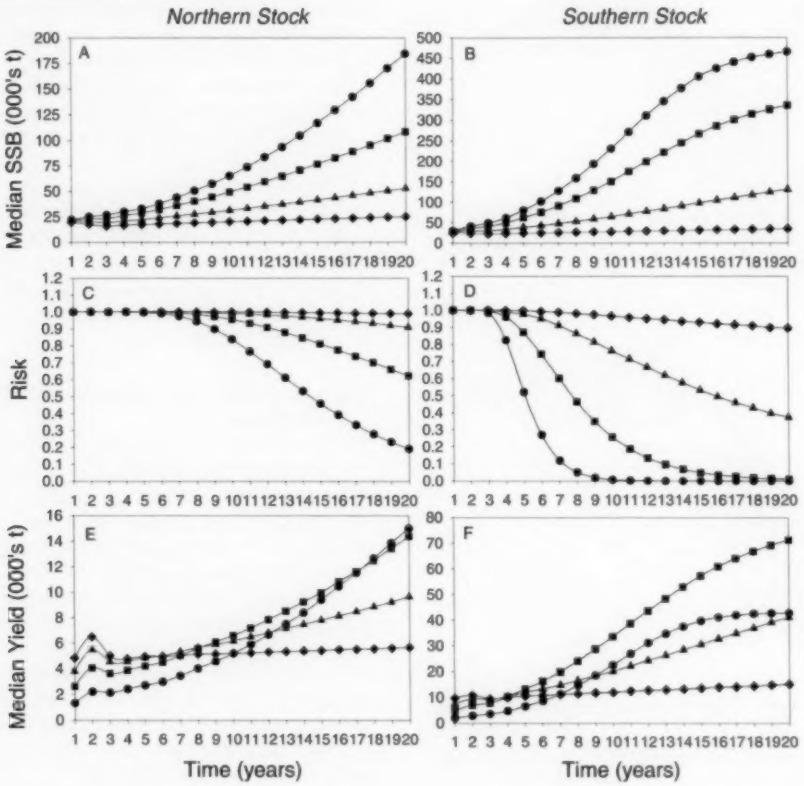
Fig. 4. Median SSB, median yield, and risk (prob($SSB_t < SSB_{50}$)) trajectories (20 years) from stochastic simulation model under the assumption of density-independent life history parameters. Individual trajectories (lines) were generated from varying constant F harvest rates. Northern stock: $F = 0.1$ (circles), $F = 0.2$ (squares), $F = 0.3$ (triangles), $F = 0.4$ (diamonds); southern stock: $F = 0.1$ (circles), $F = 0.3$ (squares), $F = 0.5$ (triangles), $F = 0.7$ (diamonds).



Uncertainty in fishery yield and SSB

The simulation model used in this analysis incorporated process error (uncertainty in stock–recruitment) and estimation error (uncertainty in stock sizes) to more realistically capture the stochastic nature of the stock's dynamics through time. We examined the uncertainty in the simulation model output in terms of the 10th and 90th percentiles (or 80% confidence intervals) of the 20-year average values of SSB and yield realizations over all constant F harvest policies studied ($F = 0.10$ to 1.0 by 0.1 increments) under the density-dependent assumption. We again note that uncertainty is these quantities is probably underestimated due to the autocorrelated nature of the stock–recruitment data. Expected average long-term yield in the northern stock at F_{max} (0.20) was 6000 t, but yield varied widely between roughly 3000 t (10%) and 10 000 t (90%) (Fig. 7A). SSB in the northern stock was also highly variable, generally ranging ±20 000 t from the long-term expected average at lower F (<0.2) (Fig. 7B). In the southern stock, while greater yield at F_{max} (0.3) and SSB might be anticipated than compared with those in the northern stock, variation in these quantities also was considerably greater. Here, expected average yield at F_{max} (0.3) reached 23 000 t, but yields also varied widely between 13 000 and 37 000 t (Fig. 7C). Also, SSB in the southern stock was highly variable, particularly for harvest policies that generated significant stock rebuilding (e.g., $F <$ 0.3), ranging from 50 000 to 200 000 t (Fig. 7D).

Discussion

In recent years, much has been done to improve the scientific advice provided to fishery managers in terms of estimating and defining risk and uncertainty (Smith et al. 1993; Rosenberg and Restrepo 1994). Despite these advances, scientific advice may still suffer from lack of biological realism which can arise from inappropriate model assumptions. One such assumption commonly used in fishery assessment models concerns static life history parameters. This assumption may be unfounded because density-dependent changes in life history parameters have been widely recognized in freshwater and marine fish populations (Alm 1959; Schaffer and Elson 1975; Trippel et al. 1995). Empirical evidence has increasingly supported ecological theory of plasticity in life history parameters (such as life span, growth, and size/age at maturation) as a dynamic response between the animal and the variable (stressful) environment in which it lives (Roff 1984; Stearns and Crandall 1984). For this reason, it is not surprising that plasticity in growth and maturation rates in numerous fish stocks have been observed in relation to the stresses of overexploitation (Trippel

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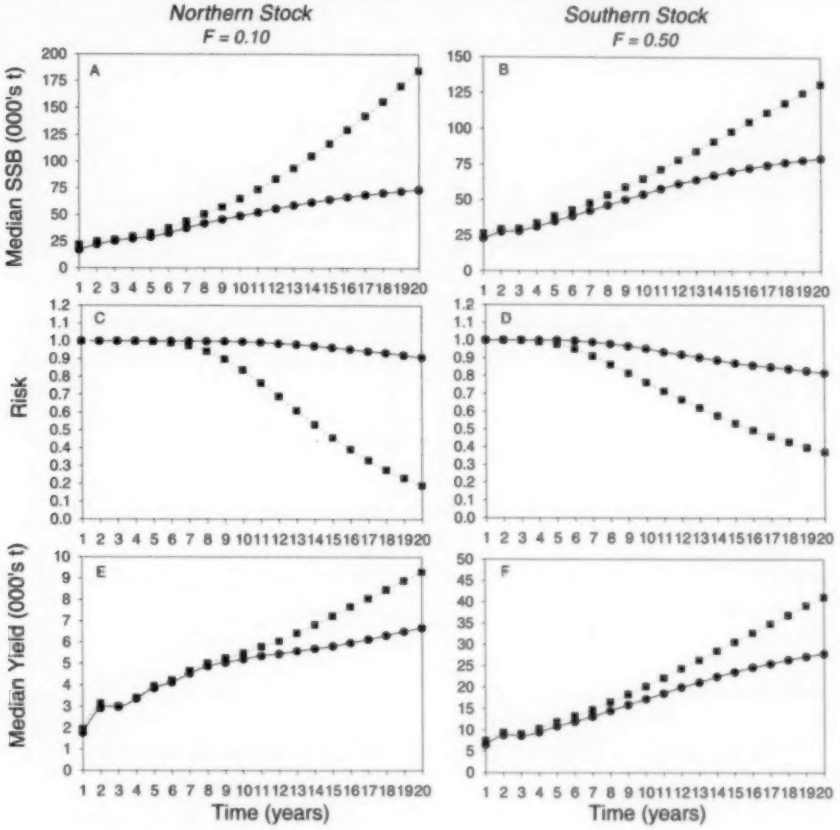
Fig. 5. Median SSB, median yield, and risk (prob($SSB_t < SSB_{50}$)) trajectories (20 years) from stochastic simulation model comparing assumptions of density-independent (broken lines) and density-dependent (solid lines) life history parameters. Trajectories shown were generated from constant F harvest rates of 0.10 and 0.50 for the northern and southern stocks, respectively.



1995). Our modeling effort was based on the premise that as population abundance increases, life history parameters (i.e., growth and maturity) of the stock would respond in a predictable compensatory manner. It should be noted, however, that an alternative hypothesis could be possible; changes in life history traits may involve genetic selection, as has been shown for some salmonids (Tipping 1991). Instead of population density, earlier age at maturity, for instance, becomes a function of changes in genotype frequency. The most obvious outcome of our analysis is that rebuilding strategies would be unaffected by changes in growth and maturation, as these would be a density-independent phenomena that are non-stationary.

While limited efforts have been generally made to incorporate this dynamic response of life history parameters into assessment models (Overholtz et al. 1991), the lack of empirical evidence to support a compensatory hypothesis should not deter fishery scientists from carefully considering the impacts of such basic assumptions on that advice. Our results indicated that model assumptions regarding static life history parameters resulted in scientific advice for the U.S. silver hake stocks that was far too optimistic. In particular, unless compensatory mechanisms including density-dependent growth and sexual maturity are accounted for, scientific advice may provide unrealistic expectations of F targets to provide rebuilding of the stocks, requiring longer rebuilding times and entailing more risk and unrealized levels of long-term sustainable yield. Overholtz et al. (1991) found similar results when evaluating the impact that density-dependent growth, maturity, and natural mortality had on providing advice for Northwest Atlantic mackerel stocks. Our analysis represents the first of its kind in evaluating the impact of compensatory responses of life history parameters on rebuilding a depleted groundfish stock in the Northwest Atlantic. In our analysis, we did not incorporate an explicit description for density-dependent natural mortality (therefore assumed static), but empirical evidence suggests increased rates of cannibalism in areas where interactions between different size components occur, particularly in the southern stock region (R.A. Rountree and M.J. Fogarty, unpublished data). Such interactions are likely to be more frequent under greater population sizes.

Additional analyses are necessary to improve the biological realism of the models and scientific advice provided to manage the silver hake stocks. Compensatory responses of natural mortality rates should be investigated and intra- and interspecific predation mortality quantified from food habits data.

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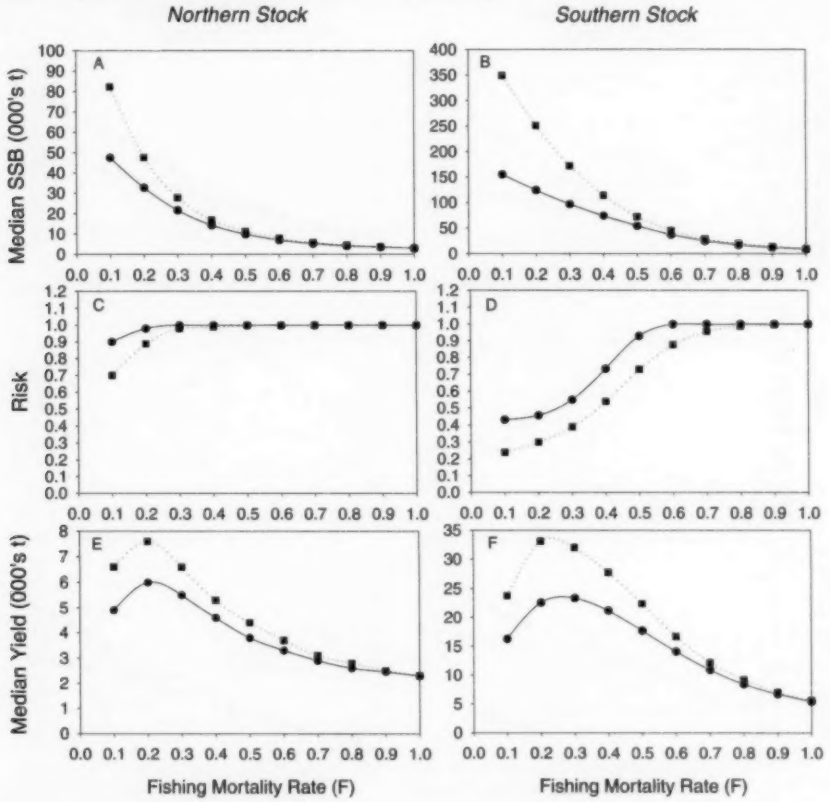
Fig. 6. Comparison of long-term average SSB, yield, and risk (prob($SSB_t < SSB_{50}$)) from stochastic simulation model with increasing constant F under different assumptions of density-independent (broken lines) and density-dependent (solid lines) life history parameters. Long-term average was taken as the average of the SSB, yield, and risk realizations over 20 years.



This information would be particularly useful in attempting to estimate an M2 component of predation mortality in the framework of a multispecies VPA. In addition, individual weights and sexual maturities should be closely monitored through fishery-independent surveys to assess their response to changing population states. Further, effects of lower mean age/size at reproduction (due to stresses of overexploitation) on fish fecundity need to be evaluated for these stocks. Viable egg production and subsequent reproductive success may be compromised under conditions associated with compensation such as reductions in egg size (Knutsen and Tilseth 1985; Buckley et al. 1991) and compression of spawning times (Lambert and Ware 1984). These factors could lead to rationalizing harvest rates that have already exhausted much of the population's compensatory capacity (Rosenberg et al. 1994).

Density dependence in life history parameters will also influence estimates of actual population status and fishing mortality based overfishing definitions. For instance, changes in these important parameters will affect population dynamics by modifying long-term sustainable levels of yield, illustrated in our simulation by large differences in long-term average levels of yield and changes in F_{max}, although the latter were not as pronounced. This is why F targets derived from yield-per-recruit analyses ($F_{0.1}$, F_{max}) would probably not be very useful, since they are based on the concept of long-term stability in life history parameters (Beverton and Holt 1957). Therefore, assessment advice generated from equilibrium yield-per-recruit models that is contingent upon these important parameters should be evaluated frequently as population abundance changes.

Overfishing definitions for the U.S. silver hake stocks are based on survival ratios estimated from stock–recruitment observations (Gabriel et al. 1989). These were estimated to be 31 and 42% of maximum spawning potential for the northern and southern stocks, respectively, and correspond to fishing mortality rates (F_{rep}) of about $F = 0.37$ (NEFSC 1990). A recent review of the overfishing definitions for U.S. Fishery Management Plans (Rosenberg et al. 1994) has, however, revised F_{rep} calculations to 0.40 and 0.70 for the northern and southern stocks, respectively. While overfishing definitions based on stock–recruitment data may be more robust with regard to assumptions concerning life history parameters, problems still may arise in determining stock status and planning for the recovery of overfished stocks. In our analysis, harvesting the stocks at these fishing mortality targets (F_{rep}) maintained SSB trajectories under the assumption of density independence. This would be expected, since these rates are based on Fs that generate sufficient recruitment to replace successive generations of spawners over time (Gabriel et al. 1989). It was apparent that more conservative harvest F policies ($F < 0.2$ and

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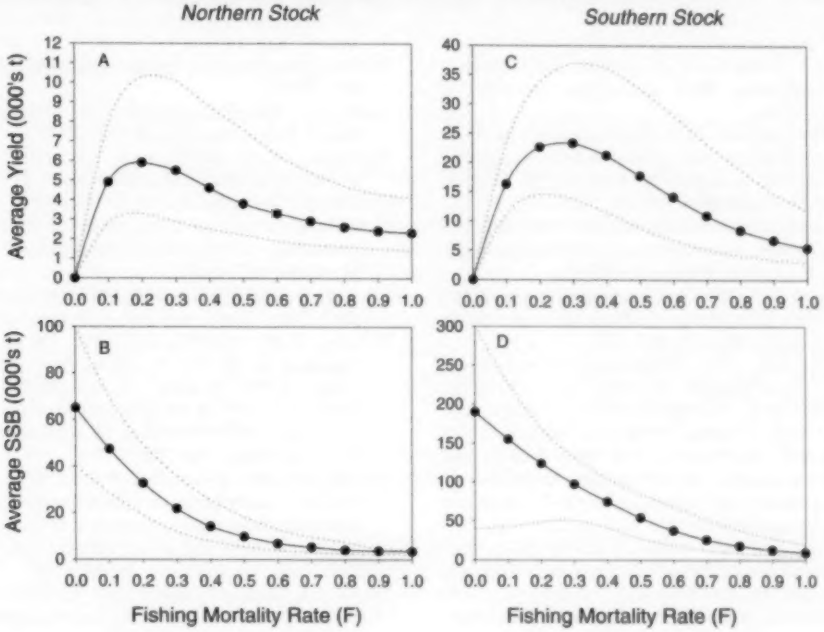
Fig. 7. Uncertainty in long-term average yield and SSB from stochastic simulation under the assumption of density-dependent life history parameters with increasing constant F. Long-term expected values (solid lines) and associated 80% confidence intervals (broken lines) were taken as the average of the 10th, 50th, and 90th percentiles of the yield realizations over 20 years.



$F < 0.5$ in the northern and southern stocks, respectively) would, however, be needed to promote stock rebuilding because the F_{rep} targets only maintained the biomass which was already in a depleted state. Perceptions regarding the status of the stocks, however, were problematic because even under these relatively low Fs, stocks did not rebuild to levels expected when density dependence in life history parameters was accounted for. In addition, more time was required to achieve stock rebuilding than expected, suggesting that planning horizons for the recovery of overfished stocks should be carefully evaluated in light of compensatory responses.

Further, unrealistic expectations of yield projections may have unforeseen economic consequences for fishery harvesters, affecting costs of capital investments and returns from variable relative prices and, in general, making planning difficult. This would be particularly true for silver hake fisheries because revenues respond to prices that are dependent on the size composition of the stock and quantities supplied (Helser et al. 1996). These factors also point to the relevance of evaluating different utility functions to define risk other than the simple threshold biomass approach used in this simulation. Megrey et al. (1994) incorporated yield into various formulations of risk within the context of a bioeconomic simulation, and Smith et al. (1993) provided other possible definitions. In large part, however, defining such utility functions expressing risk depends on a well-defined set of goals for management. One key point in our simulation results incorporating a simple biomass threshold into the definition of risk is the marked differences in the levels of risk to the stocks under the two model assumptions comparing static versus variable life history parameters. Because there is a lack of current information on population size and fishery selectivity patterns for the silver hake stocks, the simulation results should be interpreted cautiously. Nonetheless, our results clearly show the importance of accounting for compensatory responses in silver hake life history parameters in assessment and management advice.

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Postglacial fish dispersal from the Mississippi refuge to the Mackenzie River basin

L.L. Rempel and D.G. Smith

Abstract: Wisconsinan glaciation had a profound impact on fish faunas in North America, and deglaciation led to the dispersal of approximately 28 species from the Mississippi glacial refuge into the Mackenzie River basin. A hypothesized dispersal 11 500 years ago via glacial lakes Agassiz and Peace is difficult to verify and hydrologic linkage between these lakes was sporadic and short lived. Geomorphic evidence indicates that glacial Lake Agassiz drained into the Mackenzie basin via the Clearwater River, Saskatchewan, 9900 years ago and created a second opportunity for fish dispersal northward. Fish distribution data indicate a 96% similarity between Mississippi species in the Mackenzie basin and species occupying the former Agassiz–Clearwater corridor. Fifteen species dispersed into the headwaters of the Clearwater River during hydrologic linkage to Lake Agassiz and are now isolated above an 18.5 m waterfall. Previous genetic data suggest lake trout (*Salvelinus namaycush*) used the Agassiz–Clearwater corridor for two-way dispersal between Beringia and the Great Lakes basin. Lake whitefish (*Coregonus clupeaformis*) populations of Mississippi origin are distinguished by a marker allele of glucose-3-phosphate dehydrogenase, *G3PDH-1*b*, that is absent from modern populations within the former Lake Peace region and the distribution of Mississippi whitefish across Canada is best resolved by our Agassiz–Clearwater dispersal hypothesis. Our research substantially alters the interpretation of fish biogeographic patterns in Canada and generates testable hypotheses for future studies.

Résumé : La glaciation wisconsinienne a profondément marqué l'ichtyofaune d'Amérique du Nord, et le retrait glaciaire a entraîné la dispersion dans le bassin du Mackenzie d'environ 28 espèces provenant du refuge glaciaire du Mississippi. L'hypothèse d'une dispersion survenue il y a 11 500 ans par les lacs glaciaires Agassiz et Peace est difficile à vérifier, et le lien hydrologique entre ces lacs a été sporadique et de courte durée. D'après les données géomorphologiques, il y a 9900 ans, le lac Agassiz se déversait dans le bassin du Mackenzie par la rivière Clearwater (Saskatchewan), ce qui représente une deuxième possibilité de dispersion des poissons vers le nord. Les données sur la répartition des poissons révèlent un taux de similitude de 96 % entre les espèces du Mississippi présentes dans le bassin du Mackenzie et les espèces occupant l'ancien couloir Agassiz–Clearwater. Quinze espèces se sont dispersées dans le cours supérieur de la Clearwater lorsqu'elle était reliée au lac Agassiz; elles sont maintenant isolées en amont d'une chute de 18,5 m. D'après les données génétiques antérieures, le touladi (*Salvelinus namaycush*) fréquentait le couloir Agassiz–Clearwater, ce qui aurait permis une dispersion à double sens entre la Béringie et le bassin des Grands Lacs. Les populations de grand corégone (*Coregonus clupeaformis*) originaires du Mississippi se différencient par une allèle marqueur de glucose-3-phosphate déshydrogénase, *G3PDH-1*b*, qui est absent chez les populations actuelles de l'ancienne région du lac Peace, et la répartition du corégone du Mississippi au Canada s'explique de la façon la plus plausible par notre hypothèse de dispersion par le couloir Agassiz–Clearwater. Nos recherches modifient sensiblement l'interprétation des profils biogéographiques des poissons au Canada et soulèvent des hypothèses que pourront vérifier de futures études.
[Traduit par la Rédaction]

Introduction

Late Wisconsinan glaciation had a profound impact on fish fauna in northern North America, whereas prior glaciations were less extensive and probably less disruptive to fish populations (Young et al. 1994). Maximum glaciation culminated 18 ka BP (thousands of radiocarbon years before present), at which time continental ice covered most of Canada and the northern United States (Fig. 1). Fish were confined to areas of refuge during glaciation and the sequence of deglacial events produced enormous volumes of water, which gave way to temporary glacial lakes and spillways. Drainage divides were often inundated and watersheds temporarily connected by large meltwater lakes, providing opportunities for fish to disperse from the confines of refugia into newly deglaciated regions. Broad-scale patterns of fish species distributions and phylogeography resulting from these events continue to be a focus of research (e.g., Bernatchez and Dodson 1991; Foote et al. 1992; Wilson 1995). A major, unresolved issue in fish biogeography has been the postglacial dispersal of fish species from the Mississippi refuge into the Mackenzie basin.

The Mississippi was one of five refugia (the others were Beringia, Nahanni, Pacific, and Atlantic) in North America that harboured fishes during the Wisconsinan glaciation (Fig. 1). It was the most significant refuge with respect to the number of refugial species it hosted and the geographic area it

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Fig. 1. Locations of glacial refugia, modern drainage basins, and major lakes and rivers across North America. The two dispersal corridors referred to in the text are indicated.

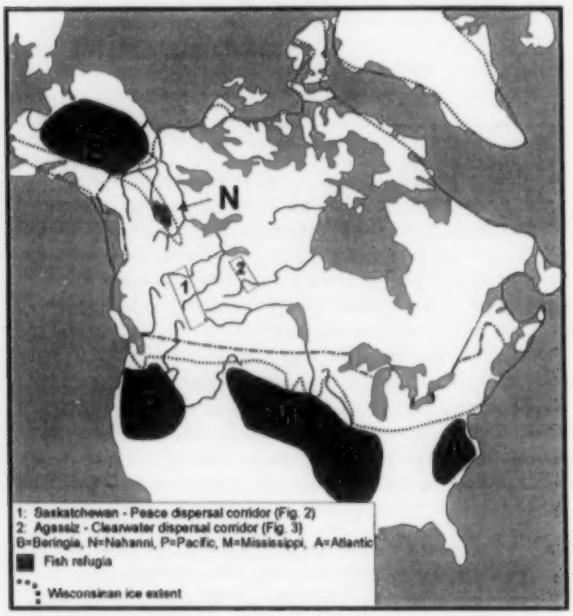
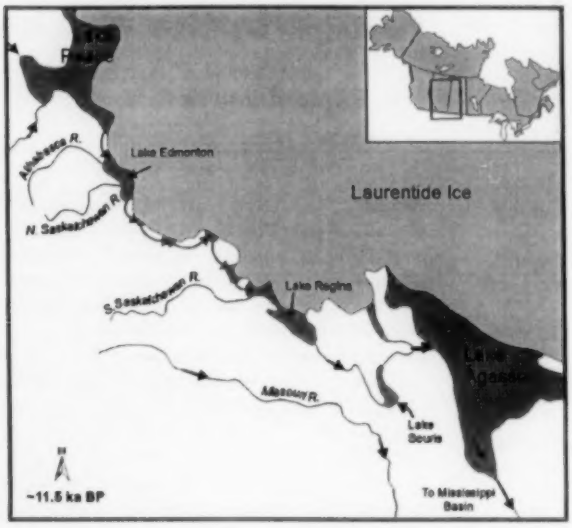


Fig. 2. The glacial Lake Saskatchewan–Peace corridor for fish dispersal at approximately 11.5 ka BP (after Dyke and Prest 1987).



recolonized (McPhail and Lindsey 1970; Crossman and McAllister 1986). Dispersal from the Mississippi refuge northward into the Nelson River basin followed the southern outlet of glacial Lake Agassiz between 12 and 11 ka BP (Lindsey and McPhail 1986). The route of dispersal beyond the Nelson basin and into the Mackenzie basin remains uncertain.

Fish dispersal at approximately 11.5 ka BP was hypothesized by McPhail (1963) via a series of temporary proglacial lakes and spillways between Lake Agassiz and the Mackenzie basin. Geomorphic evidence for the existence of these lakes was reviewed by Taylor (1960) and substantiated by St-Onge (1972). Glacial Lake Peace originally drained via Lesser Slave Lake into glacial Lake Edmonton–Leduc and outflowed into Lake Agassiz via lakes Regina and Souris (Fig. 2). This waterway, herein referred to as the Saskatchewan–Peace corridor, extended over a vast distance for less than 400 years, and its timing and connectivity is difficult to verify (Teller 1987). As a route for fish dispersal, it is questionable because of hostile and unstable environmental conditions. Kehew and Clayton (1983) argued connections between lakes Regina, Souris, and Hind were unstable and they repeatedly drained into one another in catastrophic, domino-like fashion to incise deep spillway trenches. The volume capacity of each lake basin was small relative to the size of spillways, suggesting that episodes of discharge were enormous and short lived. Large braided river deltas of coarse glacial sediment prograded into each lake basin inferring that waters were heavily laden with coarse sediment (St-Onge 1972). Recent sedimentary evidence presented by Holden (1993) suggests that Lake Edmonton was subglacial, indicating food resources were limited and waters were extremely frigid and sediment-laden. The existence of Lake Edmonton was short-lived and highly unstable, thus requiring rapid fish dispersal into Lake Peace, which eventually drained along the modern Peace River valley into the Mackenzie River by 11 ka BP (Mathews 1980).

Lake Agassiz continued to persist after the demise of lakes Edmonton and Peace, increasing to a maximum size of 350 000 km^2 by 9.9 ka BP (Teller 1987). It has been recently substantiated that a northwestern outlet of Lake Agassiz flowed into the Mackenzie basin via the Clearwater River and glacial Lake McConnell between 9.9 and 9.5 ka BP, thus affording fish a second opportunity to disperse into the Mackenzie basin (Smith and Fisher 1993; Fisher and Smith 1994; Smith 1994; Fig. 3). Several lines of evidence support the existence of environmentally benign conditions and productive aquatic habitat for fish survival. Mean summer temperatures had increased from 4°C at 11 ka BP to approximately 14°C by 9.5 ka BP in southwestern Manitoba (Ritchie 1983). Fossils of bison (*Bison* sp.) and yellow perch (*Perca flavescens*) dated at 9.7 ka BP have been collected from the Lake Agassiz basin (Stewart and Lindsey 1983; Nielsen et al. 1984) (Fig. 3). Food resources for aquatic fauna were available as pollen data indicate that the western shore of Lake Agassiz was vegetated after 11 ka BP and 76 invertebrate taxa have been uncovered from a weed-filled bog in the lake basin dated at 9.9 ka BP (Ashworth and Cvancara 1983). Fossils of 26 species of molluscs dated between 11.5 and 10 ka BP, including 5 mussel species that require fish hosts for survival, were also recovered. Within the Mackenzie basin, woodlands of spruce and birch were established by 11 ka BP (Vance 1986; MacDonald 1987). A sediment core from the glacial Lake McConnell basin contained two mollusc genera, *Gyraulis* sp. and *Pisidium* sp., and a considerable increase in organic matter after 10.1 ka BP, inferring that environmental conditions beyond Lake Agassiz and in the Mackenzie basin were also favourable for fish survival (Beierle 1996; Fig. 3).

In this paper we document the Agassiz–Clearwater corridor and its role in postglacial dispersal of Mississippi fishes into

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the Mackenzie River basin. To evaluate the potential importance of this corridor in fish biogeography, we sampled the current fish community of the Clearwater River. Distributional data of fish species across several watersheds in Canada and the Clearwater River were then compared for community similarity to substantiate geomorphic evidence of Smith and Fisher (1993) for the corridor. Previously published distribution and genetic patterns of several fish species were also examined to identify the most parsimonious route of postglacial dispersal and to clarify the importance of the Agassiz–Clearwater corridor in shaping present patterns of fish biogeography.

Materials and methods

Our study area was along the Clearwater River between Lloyd Lake, Saskatchewan, and Fort McMurray, Alberta (Fig. 4). The river drops 150 m over a distance of 295 km between these locations. Three reaches of river were identified for our study, each separated by major waterfalls (Fig. 4). The upper reach (Reach 1) consisted of low-gradient, braided channels with a broad floodplain in a virtually undefinable river valley. Smoothrock Falls marked the downstream boundary of Reach 1, and its location coincides with the confluence of the Lake Agassiz spillway. Smoothrock Falls is composed of highly resistant Precambrian Shield bedrock and has a vertical drop of 18.5 m. The former spillway valley confines the Clearwater River for its remaining 100 km with an average width of 4 km and depth of 120 m. Reach 2 was characterized by several long series of rapids and chutes. The Alberta border and Whitemud Falls marked the lower boundary of Reach 2 and the beginning of Reach 3, which extended to Fort McMurray. Whitemud Falls is eroded into Devonian limestone and its 30-m vertical drop extends over a series of major steps. Channel substrate along the entire length of river is predominantly coarse sand with fine sand and pockets of macrophytes in backwater side channels. No quantitative analyses of channel substrate were completed.

The fish community in the Clearwater River was sampled during July and August, 1993. Thirty-six sites were sampled and site selection was based on accessibility, river gradient, and substrate conditions. A river seine net (0.6-cm mesh), gill net (2 × 46 m of 5-cm mesh), and angling were used for sampling. Gill nets were seldom used because of the swift gradient and organic debris, which clogged and damaged the net. The ease and convenience of seine netting allowed thorough sampling in shallow-water habitats. In regions of high gradient, sampling was restricted to angling. Fish were identified in the field following Nelson and Paetz (1992). Voucher specimens of all species were overdosed with MS-222, preserved in 10% formalin, and given to experts for identification (Dr. J.D. McPhail, Department of Zoology, University of British Columbia, Vancouver, and W.E. Roberts, Museum of Zoology, University of Alberta, Edmonton). All other fish were identified, counted, and released. Fish collection data from previous investigations of the Clearwater River were included for analysis (Jones et al. 1978; Tripp and McCart 1979; Atton and Merkowsky 1983).

The similarity of fish communities in the Clearwater River and those in drainages overlapping the Saskatchewan–Peace and Agassiz–Clearwater dispersal corridors was measured using Jaccard's index of faunal similarity which compares presence–absence data of species between two drainages (Krebs 1989).

Results

Twenty-seven of 28 species in the Clearwater River appear to have originated from the Mississippi glacial refuge (Table 1). Mountain whitefish (*Prosopium williamsoni*) is of Pacific refugial origin, dispersing across the Continental Divide and into the Mackenzie basin following glaciation (Lindsey and McPhail 1986). Species in the Clearwater River that occupied multiple refugia are believed to be of Mississippi origin based on previous biogeographic studies (Table 1). Fifteen of 28 species were collected in Reach 1 above Smoothrock Falls. The high-gradient flow downstream of the Lake Agassiz confluence limited sampling opportunities in Reach 2, and only 10 species were collected along this 35-km stretch of river. Of these 10 species, all but mountain whitefish were also collected upstream in Reach 1. Reach 3 of the Clearwater River had been sampled by previous studies, and a total of 26 fish species have been identified (Jones et al. 1978; Tripp and McCart 1979; Atton and Merkowsky 1983).

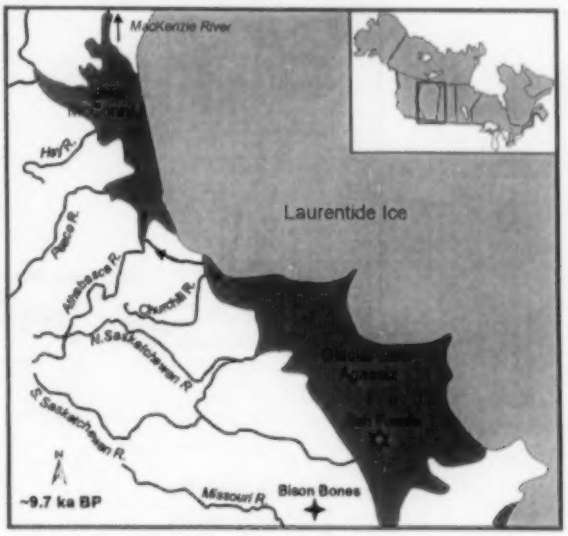

Fig. 3. The glacial Lake Agassiz–Clearwater River corridor for fish dispersal at approximately 9.7 ka BP (after Smith and Fisher 1993). See text for details of bison bones and fish fossils.

Twenty-eight species in the Mackenzie River basin originate from the Mississippi refuge (Table 2). Twenty-seven of these 28 species occur in the Clearwater River and Nelson basin, and 25 are found in the Churchill basin. Jaccard's index of similarity between the Mackenzie basin and both the Nelson basin and Clearwater River was 0.96. The Churchill basin was highly similar to the Mackenzie (0.89). The upper and lower Peace rivers overlap the Saskatchewan–Peace corridor, and six of the Mackenzie basin species are absent from the lower Peace River. Nineteen of the 28 species are absent from the upper Peace River. Jaccard's indices between these rivers and the Mackenzie basin were 0.79 and 0.32, respectively.

Discussion

A century ago, Upham (1895) speculated that Lake Agassiz assumed a northern outlet and temporarily drained into the Mackenzie basin. The Clearwater River was hypothesized as the spillway corridor of Lake Agassiz by Elson (1967) but only recently has geomorphic evidence been available to support this claim (Smith and Fisher 1993; Fisher and Smith 1994). The results of our study are congruent with geomorphic

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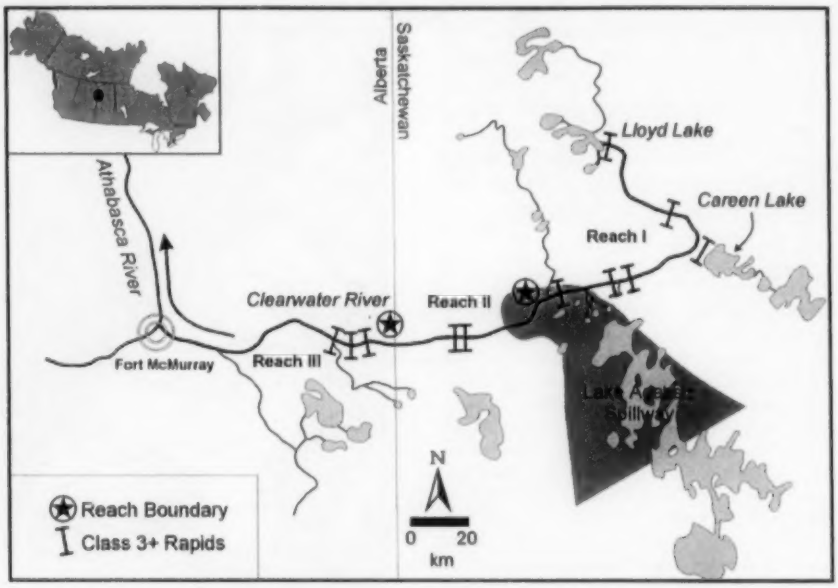
Fig. 4. Study area along the Clearwater River between Lloyd Lake, Saskatchewan, and Fort McMurray, Alberta. The shaded area delineates where Lake Agassiz flowed into the Clearwater River between 9.9 and 9.5 ka BP.



information and suggest that up to 28 fish species used the Agassiz–Clearwater corridor to disperse from the Mississippi refuge into the Mackenzie basin. The fish community of the Clearwater River is dominated by species of Mississippi origin (96%). Fifteen species were collected in Reach 1 of the Clearwater River above Smoothrock Falls, which has a vertical drop of 18.5 m.

Smoothrock Falls may serve as a dispersal barrier for many fish species and opportunities for dispersal into Reach 1 were likely facilitated by discharge from Lake Agassiz. The distribution of mountain whitefish supports this hypothesis, as its postglacial colonization of the Clearwater River from the Pacific refuge has been restricted to reaches downstream of Smoothrock Falls. Mountain whitefish is adapted to cold-water, high-gradient environments (Lindsey and McPhail 1986) but has not surmounted the waterfall. Reach 1 above Smoothrock Falls is characterized by low-gradient flow and backwater habitats that are the preferred conditions for many of the 15 species in Reach 1, all of which are of Mississippi origin. The most reasonable explanation is that the dispersal of these species occurred during hydrologic linkage between Lake Agassiz and the Clearwater River.

Fish dispersal via the Agassiz–Clearwater corridor is also supported by findings of Bernatchez and Dodson (1991), where distribution patterns of fish species overlapping areas of glacial refuge and those formerly occupied by glacial lakes and spillways indicated most probable dispersal routes. The Nelson and Churchill basins overlap the corridor and their similarity to the assemblage of Mississippi species in the Mackenzie basin is very high (0.96 and 0.89, respectively). Lake Agassiz extended into the Churchill basin only after the Saskatchewan–Peace corridor had closed, yet the Churchill basin is more similar to the Mackenzie basin than is the upper Peace River to the Mackenzie (0.32) within the former Saskatchewan–Peace corridor. The lower Peace River was still glaciated at the time of the Saskatchewan–Peace corridor and is tributary to the Mackenzie River with no major flow obstacles along its course to inhibit fish movement, thus accounting for its high faunal similarity with the Mackenzie (0.79). In contrast, few fish species in the Mackenzie basin are also above the Peace River canyon of the upper Peace River, which was temporarily inundated by glacial Lake Peace 11 ka BP (Mathews 1980). Moreover, populations of several fish species above and below the canyon are morphologically distinct (Lindsey and McPhail 1986).

Previously published data also provide support for fish dispersal via the Agassiz–Clearwater corridor. These data involve species that occupied more than one glacial refuge, and modern populations have retained distinguishing morphometric or biochemical traits that allow their refugial origin to be determined. First, mitochondrial DNA (mtDNA) diversity in lake trout (*Salvelinus namaycush*) indicate two clades of Bering origin dispersed across central Canada and into the Great Lakes basin (Wilson 1995). Wilson (1995) recognized the Agassiz–Clearwater corridor as the probable dispersal route for these Bering groups. High proportions of lake trout with Mississippi mtDNA lineage were collected from the Nelson and Churchill basins within the Agassiz–Clearwater corridor, and in Lake Athabasca, Mackenzie basin. Populations in the lower Peace River, however, were predominantly of Bering origin and those in Swan Lake, Alberta, within the former Saskatchewan–Peace corridor were entirely of Bering origin. The most parsimonious explanation for these phylogeographic patterns is the Agassiz–Clearwater corridor serving as a two-way dispersal corridor for lake trout between Beringia and the Mississippi refuge.

Second, northern flow of glacial Lake Agassiz into the Mackenzie basin was hypothesized by Black (1983) to resolve

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Table 1. Refugial origins and occurrence of fish species in the Clearwater River for reaches identified in Fig. 4.

Species	Reach 1	Reach 2	Reach 3	Refuge
Goldeye (*Hiodon alosoides*)	—	—	3	M[a]
Cisco (*Coregonus artedi*)	2	—	3	M[a]
Lake whitefish (*Coregonus clupeaformis*)	1, 2	1	3	M[b]
Shortjaw cisco (*Coregonus zenithicus*)	—	—	3	M[a]
Mountain whitefish (*Prosopium williamsoni*)	—	1	3	P[a]
Lake trout (*Salvelinus namaycush*)	1, 2	—	—	M[c]
Arctic grayling (*Thymallus arcticus*)	1, 2	1	3	M[d]
Northern pike (*Esox lucius*)	1, 2	1	1, 3	M[d]
Lake chub (*Couesius plumbeus*)	2	—	1, 3	M[e]
Brassy minnow (*Hybognathus hankinsoni*)	—	—	4	M[a]
Flathead chub (*Platygobio gracilis*)	—	—	3	M[a]
Emerald shiner (*Notropis atherinoides*)	—	—	2	M[a]
Spottail shiner (*Notropis hudsonius*)	1, 2	1	1, 2	M[a]
Northern redbelly dace (*Phoxinus eos*)	—	—	1	M[a]
Finescale dace (*Phoxinus neogaeus*)	—	—	3	M[a]
Fathead minnow (*Pimephales promelas*)	—	—	3	M[a]
Longnose dace (*Rhinichthys cataractae*)	—	1	3	M[e]
Pearl dace (*Margariscus margarita*)	—	—	3, 4	M[a]
Longnose sucker (*Catostomus catostomus*)	1, 2	1	1, 3	M[e]
White sucker (*Catostomus commersoni*)	1, 2	—	3	M[a]
Trout-perch (*Percopsis omiscomaycus*)	2	1	1, 3	M[a]
Burbot (*Lota lota*)	1, 2	1	1, 3	M[e]
Brook stickleback (*Culaea inconstans*)	—	—	3	M[a]
Ninespine stickleback (*Pungitius pungitius*)	1, 2	1	—	M[d]
Yellow perch (*Perca flavescens*)	1, 2	—	3	M[a]
Walleye (*Stizostedion vitreum*)	1, 2	1	1, 3	M[a]
Slimy sculpin (*Cottus cognatus*)	1, 2	—	3	M[d]
Spoonhead sculpin (*Cottus ricei*)	—	—	4	M[a]

Note: Source of collection data is indicated and the refugial origin of each species with the corresponding literature reference is given (refuge: M, Mississippi; P, Pacific). Species in bold occupied multiple refugia and the cited reference provides the refugial origin of Clearwater populations. Data sources for the three reaches are (1) present study; (2) Atton and Merkowsky (1983); (3) Tripp and McCart (1979); (4) Nelson and Paetz (1992); and (–) not present.

[a]Crossman and McAllister (1986).
[b]Foote et al. (1992).
[c]Wilson (1995).
[d]Lindsey and McPhail (1986).
[e]McPhail and Lindsey (1970).

the distribution pattern of *Cystidicola stigmatura*, a parasitic nematode that inhabits the swimbladder of lake trout The species survived glaciation in the Mississippi refuge and is distributed from the Great Lakes into the Mackenzie basin (Black 1983). Distribution evidence suggests the parasite dispersed from the Mississippi after 10.9 ka BP into the Great Lakes basin and then migrated north with lake trout via Lake Agassiz into the Mackenzie basin. *Cystidicola stigmatura* is absent from the Saskatchewan–Peace corridor region, but its distribution overlaps the Agassiz–Clearwater corridor and is best explained by this route of dispersal. In addition, *C. stigmatura* is dependent on both the presence of lake trout and *Mysis relicta*, the opossum shrimp, to complete its life cycle. Allozyme data from *M. relicta* indicates that populations in the former Lake Agassiz basin and those in the Mackenzie basin are genetically similar and of Mississippi origin, which supports the ability of *C. stigmatura* to disperse into northern Canada (Väinölä et al. 1994).

A third line of evidence for fish dispersal via the Agassiz–Clearwater corridor is from lake whitefish of Mississippi origin that are biochemically distinguished from other refugial origins by a high frequency of an allele of glucose-3-phosphate dehydrogenase, *G3PDH-1*b* (Foote et al. 1992). The frequency of this allele in populations from Lake Athabasca and Great Slave Lake in the Mackenzie basin is 0.4 and 0.36, respectively. The *G3PDH-1*b* allele is absent from populations occupying the Saskatchewan–Peace corridor region in the Athabasca and Peace rivers and is low in lake whitefish (*Coregonus clupeaformis*) from Lesser Slave Lake (0.03). A Nahanni glacial refuge is hypothesized for these populations, and there appears to have been little introgression among Mississippi and Nahanni populations. Lake whitefish in the Mackenzie basin, therefore, most likely dispersed via the Agassiz–Clearwater corridor from the Mississippi refuge. The results of Foote et al. (1992) are concordant with biogeographic data from Franzin and Clayton (1977) of the *G3PDH* b^2 allele

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Table 2. Distribution of fish species in the Mackenzie River basin that originate from the Mississippi refuge.

Species	Clearwater	Churchill	Nelson	Upper Peace	Lower Peace
Goldeye (*Hiodon alosoides*)	+	+	+	—	+
Cisco (*Coregonus artedi*)	+	+	+	—	—
Lake whitefish (*Coregonus clupeaformis*)	+	+	+	+	+
Shortjaw cisco (*Coregonus zenithicus*)	+	+	+	—	—
Lake trout (*Salvelinus namaycush*)	+	+	+	+	+
Arctic grayling (*Thymallus arcticus*)	+	+	—	+	+
Northern pike (*Esox lucius*)	+	+	+	—	+
Lake chub (*Couesius plumbeus*)	+	+	+	+	+
Brassy minnow (*Hybognathus hankinsoni*)	+	—	+	—	+
Flathead chub (*Platygobio gracilis*)	+	—	+	—	+
Emerald shiner (*Notropis atherinoides*)	+	+	+	—	—
Spottail shiner (*Notropis hudsonius*)	+	+	+	—	—
Northern redbelly dace (*Phoxinus eos*)	+	+[a]	+	—	+
Finescale dace (*Phoxinus neogaeus*)	+	+	+	—	+
Fathead minnow (*Pimephales promelas*)	+	+	+	—	+[b]
Longnose dace (*Rhinichthys cataractae*)	+	+	+	+	+
Pearl dace (*Margariscus margarita*)	+	+	+	—	+
Longnose sucker (*Catostomus catostomus*)	+	+	+	+	+
White sucker (*Catostomus commersoni*)	+	+	+	+	+
Trout-perch (*Percopsis omiscomaycus*)	+	+	+	—	+
Burbot (*Lota lota*)	+	+	+	+	+
Brook stickleback (*Culaea inconstans*)	+	+	+	—	+
Ninespine stickleback (*Pungitius pungitius*)	+	+	+	—	—
Iowa darter (*Etheostoma exile*)	—	+	+	—	+
Yellow perch (*Perca flavescens*)	+	+	+	—	+
Walleye (*Stizostedion vitreum*)	+	+	+	—	+
Slimy sculpin (*Cottus cognatus*)	+	+	+	+	+
Spoonhead sculpin (*Cottus ricei*)	+	+	+	—	+

Note: Drainages listed are those overlapping with the former Saskatchewan–Peace and Agassiz–Clearwater dispersal corridors. Sources of distribution information were Crossman and McAllister (1986) for the Churchill and Nelson basins and (McPhail and Carveth 1993) for the Peace River; (+) present; (—) not present.

[a]Introduced (Crossman and McAllister 1986).

[b]Introduced (McPhail and Carveth 1993).

(previous nomenclature for the *G3PDH-1*b* allele), as well as geographic variation in modal gill raker counts from whitefish (Lindsey et al. 1970).

Lastly, Bernatchez and Dodson (1991) employed restriction-fragment length polymorphisms to evaluate phylogenetic relationships in mtDNA of lake whitefish. Populations of Mississippi origin are distributed across Canada, from Labrador to the Mackenzie basin. Fish dispersal into eastern Canada from Lake Agassiz occurred after 9.5 ka BP, immediately following closure of the Agassiz–Clearwater corridor and 2000 years later than the Saskatchewan–Peace corridor. The principle of vicariance biogeography distinguishes biogeographic patterns in fishes that are congruent in space and time (Mayden 1988). If phylogenetic relationships of fishes are congruent with the timing and drainage patterns developed during deglaciation, than the vicariance principle is supported. The Agassiz–Clearwater corridor for the widespread dispersal of Mississippi lake whitefish between Labrador and Great Slave Lake supports the vicariance hypothesis; its timing with opportunities for northern dispersal into the Mackenzie basin and eastern dispersal into the Great Lakes basin, and its spatial connectivity to these locations are congruent and more reasonable than the Saskatchewan–Peace corridor.

Our research substantially alters the understanding of events associated with postglacial fish dispersal in Canada. This information is important to the interpretation of biogeographic and evolutionary patterns of fish species as genetic and morphological studies only reveal current biogeographic patterns. A knowledge of geomorphic events that have influenced these patterns is critical to their correct interpretation and to develop testable hypotheses for future research. Further biochemical studies examining the genetic relatedness of sympatric populations of widely distributed fish species will help clarify the importance of the Agassiz–Clearwater dispersal corridor.

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Effects of ration on the maturation and fecundity in captive Atlantic herring (*Clupea harengus*)

Y. Ma, O.S. Kjesbu, and T. Jørgensen

Abstract: We examined the effects of amount of food on vitellogenic oocyte size and number (fecundity) between two experimental groups of Atlantic herring (*Clupea harengus*) kept in separate but identical compartments of a large seawater tank. Growth and maturation were monitored for 1.5 years. Each group of 200 fish was fed exclusively on krill. At the end of the experiment, a high-ration group, fed 12 mg·g fish^{-1}·day^{-1}, had similar weights-at-length as wild specimens of the same stock. A low-ration group fed about half the amount of the high-ration group had significantly lower weights-at-length. Ration had no effect on oocyte size and relative potential fecundity (oocytes per gram), but mean potential fecundity (oocytes per fish) of the low-ration fish was 26% lower than that of the high-ration fish. The relative intensity of early stages of atresia was quantified histologically. Atresia is a common phenomenon in Atlantic herring and increases markedly with suboptimal feeding. Mean relative atretic intensities were estimated at about 3 and 6% for the high- and low-ration groups, respectively, but with large intragroup variation. Modeled realized fecundity (spawned eggs per fish) indicated a 9 and 40% reduction compared with the measured potential fecundities, respectively.

Résumé : Nous avons étudié les effets de la quantité de nourriture sur la taille et le nombre (fécondité) d'oocytes vitellogènes chez deux groupes expérimentaux de hareng (*Clupea harengus*) maintenus dans deux compartiments séparés mais identiques d'un grand réservoir d'eau de mer. Nous avons suivi la croissance et la maturation des poissons durant 1,5 ans. Chaque groupe de 200 poissons était nourri exclusivement de krill. À la fin de l'expérience, les poissons du groupe ayant reçu une forte ration alimentaire, soit 12 mg·g de poisson^{-1}·jour^{-1}, avaient des poids selon la longueur similaires à ceux de poissons sauvages du même stock. Les poissons du groupe ayant reçu une ration alimentaire faible (environ la moitié moins de celle de l'autre groupe) montraient des poids selon la longueur significativement inférieurs. La ration n'a pas eu d'effet sur la taille des oocytes et la fécondité potentielle relative (oocytes par gramme), mais la fécondité potentielle moyenne (oocytes par poisson) des poissons à faible ration a été de 26% inférieure à celle des poissons à forte ration. L'intensité relative des premiers stades d'atrésie a été quantifiée de façon histologique. L'atrésie est un phénomène courant chez le hareng et son intensité s'accroît de façon marquée quand l'alimentation est sous-optimale. Les intensités relatives moyennes de l'atrésie ont été estimées à environ 3 et 6% dans les groupes à forte ration et à faible ration, respectivement, mais il y avait une grande variation intra-groupe. La fécondité réalisée modélisée (oeufs pondus par poisson) a montré une réduction de 9 et 40%, respectivement, par rapport aux fécondités potentielles mesurées.
[Traduit par la Rédaction]

Introduction

Environmental conditions and nutritional status may have strong modifying effects on maturation and fecundity in fish (Wootton 1990). Most studies on the relationship between reproductive output and resulting abundance of progeny are based on theoretical models assuming egg production to be directly proportional to spawning stock biomass, an assumption seldom verified in the field (Cook and Armstrong 1986). These efforts have generally met with little success in explaining the observed variability in recruitment (Hilborn and Walters 1992). Several studies recently reviewed by Trippel et al. (1997) have pointed to the importance of maternal status for the resulting number and survival of eggs and larvae and ultimately for recruitment to the stock.

There are a number of field studies on herring reproduction in the literature, but very few experimental ones. Feeding captive Pacific herring (*Clupea pallasi*) in the later maturity stages advanced spawning time and likely increased egg weight, while starvation led to lower fecundity (Hay et al. 1988). For wild populations, Winters and Wheeler (1994) found that length-specific weight of Northwest Atlantic herring (*Clupea harengus*) varied significantly among years, while growth in length was more stable. Ovary weight was positively correlated with interannual variation in fish condition which appeared to be strongly density dependent. No clear density-dependent effect on reproductive investment has yet been shown for Northeast Atlantic herring (Almatar and Bailey 1989; Bailey and Almatar 1989). These articles give, however, examples of substantial annual changes, i.e., by a factor of up to 1.75 over a period of 10 years, in relative fecundity (i.e., per unit body weight). Tanasichuk and Ware (1987) underlined variation in ambient water temperature 2–3 months before

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Fig. 1. Temperature regime during the experiment.

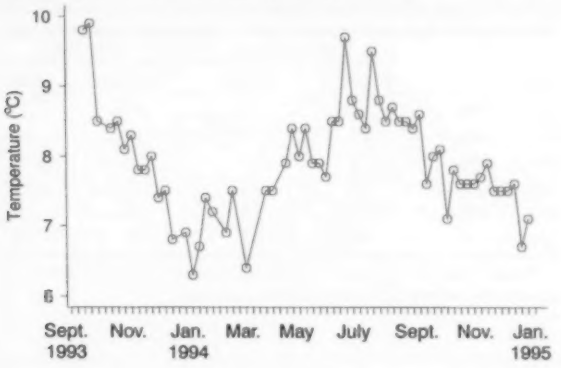
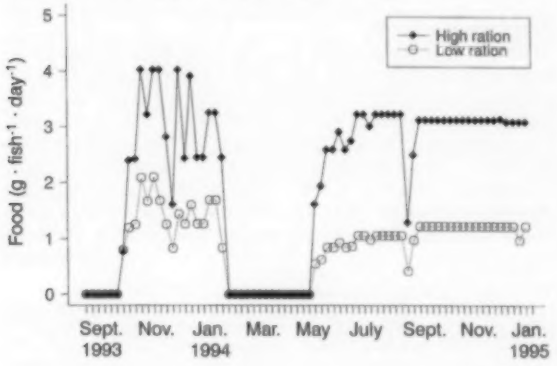


Fig. 2. Food regime during the experiment. The values given refer to weekly averages and wet weight after thawing.



spawning as important for fecundity and egg size in Pacific herring, being positively and negatively influenced, respectively. This effect was, however, not found in Atlantic herring (Bradford and Stephenson 1992).

The demonstrated effects of food supply on maturation and fecundity in fish raise the following question concerning the causal mechanisms: does food supply modulate fecundity by affecting female growth rate/condition or by affecting the rate of resorption of developing oocytes (atresia) during the maturation cycle, as suggested by many authors (e.g., Hunter and Macewicz 1985; Hay et al. 1988). Even though atresia has often been mentioned as one of the determinants of fecundity in fish, only a very limited number of quantitative studies on the incidence of atresia at various stages of oocyte development have been undertaken (Witthames and Greer Walker 1995: wild sole (*Solea solea*)). To our knowledge, no such data have yet been published for herring.

This paper presents the results of a controlled laboratory study of a local population of spring-spawning Atlantic herring. The experiment lasted 1.5 years and covered a complete maturation cycle. In a series of pilot studies, we established the daily ration that produces fish of normal condition. Then, based on this ration size, we set two experimental rations to determine how amount of food affects fish growth, maturation (vitellogenic oocyte size), level of atresia, and fecundity. In subsequent analysis of collected ovaries, special emphasis was placed on methodological aspects including precision and accuracy in fecundity estimation and intraovarian variation in oocyte size and density. Finally, we quantified histologically the intensity of vitellogenic atresia hypothesized to be an important regulatory mechanism of egg production in Atlantic herring.

Material and methods

Experimental protocol

The Atlantic herring used in the experiment were caught by purse seine on August 31, 1993, at Foldnes, west of Bergen, Norway, and transferred to a 30-m^3 outdoor concrete tank at the Institute of Marine Research. The tank was divided vertically by a fine-meshed net into two compartments, each 15 m^3. The compartments were stocked randomly with 199 and 193 fish, corresponding to the high- and low-ration experimental group, respectively. Due to initial mortality caused by scale loss/injuries, these numbers were reduced to 184 and 167, respectively. No natural mortality was observed later in the experiment. Sex ratio established at the completion of the experiment showed 52 and 50.5% females, respectively. The water supply to the tank was 200 L·min^{-1} provided from an intake at 120 m depth in the fjord. Temperature was measured weekly (Fig. 1).

After 6 weeks of adapting to the captive conditions, the fish were handled food. Each group was fed for about half an hour during daytime from Monday to Friday. In 1994, feeding was completely stopped from February 7 to May 15, likely including the duration of the local Atlantic herring spawning season (A. Slotte, Institute of Marine Research, Bergen, Norway, unpublished data). This was done to reflect the wild situation, where spawning Atlantic herring are assumed to feed very little (Henderson and Almatar 1989). The Atlantic herring were fed exclusively on krill, mainly *Meganyctiphanes norvegica*, collected and immediately frozen (–20°C) during cruises in the northeastern Norwegian Sea. Before use, the food was thawed. Temporal changes in energy content measured as lipids were noticed (S. Meier, University of Bergen, Bergen, Norway, unpublished data), but the two experimental groups received in every case food of identical origin.

Ration sizes (Fig. 2) were establish to produce fish of either good or poor condition. The high ration was about 3 g $fish^{-1}$·day^{-1} based on the results of two pilot studies, each 6 months long, aimed at producing fish with a condition typical of the wild, local population. The low ration was initially set at half of this amount from an experiment with Atlantic cod (*Gadus morhua*) (Kjesbu and Holm 1994) but reduced to one third after the first spawning season in spring 1994 to increase the difference in condition between the two groups. This low ration was slightly increased from September 6, 1994, onwards, as several fish appeared to be in a very poor condition. Thus, the low-ration fish were fed at 38% of the high ration during the last 4 months of the experiment. The overall yearly (1994) mean ration (i.e., including the 14-week starvation period) was 2.1 and 0.8 g $fish^{-1}$·day^{-1} for the high- and low-ration groups, respectively.

Samples of Atlantic herring from the two groups were collected at the start of the experiment, at intervals four times later on, and when the experiment was ended (Table 1). Total length (TL) was measured to the nearest 0.1 cm and whole-body wet weight (W) to the nearest 0.1 g. Gonad, liver, and viscera for both males and females were removed and weighed to the nearest 0.1 g and all ovaries preserved in 3.6% phosphate-buffered formaldehyde. For purposes of comparison, parallel measurements were made on wild Atlantic herring caught in early February 1996 in the same geographical area where the experimental fish were caught.

Seasonal changes in gonadal development were described using the gonadosomatic index (GSI), i.e., $10^4 \cdot W_g/TL^3$, where W_g is the wet weight of the fresh gonad. The hepatosomatic index (HSI) was used for describing the relationship between liver weight (W_h) and TL,

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i.e., $10^4 \cdot W_h/TL^3$. Somatic condition factor (SCF) was defined as $10^2 \cdot (W - W_g)/TL^3$.

Ovarian homogeneity

To study if developing oocyte size varied systematically among different locations of the ovary, three subsamples were taken from four different locations in the ovary (anterior, middle, and posterior part of the right lobe and middle part of the left lobe). For each subsample, 40 developing oocytes were measured under a binocular microscope (see below). The samples were taken from six individuals of the high-ration group in stages 3, 4, and 5 based on the Hjort maturity scale (Bowers and Holliday 1961). The data were analyzed using a nested ANOVA, and the analysis was run separately for each fish. The model was

$$(1) \qquad d_{ijk} = \mu + \mathrm{loc}_i + s_{ij} + \varepsilon_{ijk}$$

where d_{ijk} is the diameter of the kth oocyte of the jth subsample at location i, μ is the overall mean oocyte diameter, loc_i is the effect of the ith location, s_{ij} is the effect of the jth subsample at the ith location, and ε_{ijk} is the error term. Partition of the variance was obtained by the varcomp function in S-Plus, using the maximum likelihood estimation method (Venables and Ripley 1994). The partition of the variance in measured oocyte diameter showed a consistent trend for all fish studied; about 80–90% of the variance was among oocytes from the same subsample, and the remaining variance was equally distributed among locations and subsamples. Thus, the variation in oocyte size among different locations of the ovary was marginal compared with the variation in size of neighboring oocytes, and for the estimation of mean oocyte diameter, it was decided to take one subsample from each ovary. For the purpose of consistency, this subsample was always taken from the middle part of the right lobe.

Oocyte size in whole-mount preparations

Developing oocyte diameter was used as a criterion of maturity status. Using a binocular microscope (50×, precision ±10 μm), the diameter was taken as the average of the shortest and the longest axes of the oocyte. A pilot study was undertaken to test for the number of cells required to be analyzed to get reliable descriptive statistics. For each of three fish selected from the January 1995 samples, 100 vitellogenic oocytes were initially measured. Bootstrapping, i.e., 2000 iterations and bias- and acceleration-corrected percentiles (Efron and Tibshirani 1993), was then used to estimate 95% confidence intervals of the estimated mean diameter for different numbers of oocytes included. The interval width for all three fish studied decreased only marginally with sample sizes above 30–40 oocytes. Consequently, a conservative sample size of 40 was adopted for the developing oocyte diameter measurements in the main study.

Maximum previtellogenic oocyte diameter, thought to reflect minimum developing oocyte diameter, was also determined under the binocular microscope. Twelve high-ration fish were selected according to the mean diameter of developing oocytes in an effort to cover the different Hjort stages from 2–3 to 5. From each chosen ovary, besides 40 randomly selected developing oocytes, 10 of the most advanced previtellogenic oocytes were measured. Any abnormally distorted oocytes were excluded.

Histological examination of oocyte size and atresia

Before sectioning, the ovaries were dehydrated (90%) with ethanol and embedded in Historesin. Cross sections 4 μm thick were made with a metal knife. The sections were stained with toluidine blue and dried before being mounted on glass slides with Eukitt. The sections were then analyzed with an image analyzer (Olympus Micro Image) with 40 and 100× magnification for counting and measuring, respectively.

For the purpose of (*i*) contrasting measurements made of previtellogenic oocytes under the image analyzer with similar measurements made on the same fish under the binocular microscope and (*ii*) a similar comparison for vitellogenic oocytes, the following series of calibration tests were performed. Three fish from each ration group were used for determining the maximum previtellogenic oocyte size histologically; the circumferences of 10 of the most advanced, normal-looking previtellogenic oocytes were measured and the diameter calculated assuming the cell to be spherical. Only oocytes whose nucleus was sectioned were measured. Similarly, the circumferences of 40 randomly chosen developing oocytes (or the total number present if less than 40) were measured and used to estimate the corresponding diameter for a total of 43 females (both groups) sampled at various times of the year.

During oocyte development, some vitellogenic oocytes might be resorbed and are called atretic. By using the present potential fecundity estimation method, including counting of vitellogenic oocytes under the binocular microscope (detailed below), any early atretic oocytes were most likely included along with normal ones due to the difficulty in distinguishing these two types. More specifically, those that microstructurally would be characterized by showing a disrupted, but still peripheral, chorion and the granulosa cells of the surrounding follicle layer having just begun to invade the oocytic cytoplasm, i.e., the early α-atretic stage (Hunter and Macewicz 1985). To correct for these possible examples of atresia during further estimation of fecundity, histological cross sections were prepared to estimate the relative intensity of early α-stage atresia. This intensity was defined as the percentage of early α-stage atretic oocytes relative to the sum of normal vitellogenic and early α-stage atretic oocytes. The ovaries were selected from the high- and low-ration fish sampled in January 1995, 20 and 15 ovaries, respectively. To indicate the level of precision of the method chosen, three of the fish in different developmental stages from each group were first analyzed extensively. The number of vitellogenic and early α-stage atretic oocytes in fixed-size, nonoverlapping fields (snapshots from a video camera) were counted. Subsequent use of bootstrapping (described above) demonstrated that the number of fields needed to obtain stable estimates and acceptable precision varied considerably among fish. Also, the actual size of sections and thereby the number of fields varied notably due to the variation in ovarian size. It was thus decided to base the estimates of the relative intensity of early α-stage atresia on counts for the whole area of the section.

Table 1. Sampling dates and number of fish sampled by sex from each of the two experimental groups.

	High ration		Low ration	
Date	Females	Males	Females	Males
Sept. 1, 1993	4[a]	7[a]		
Feb. 9, 1994	27	16	17	18
May 4, 1994	7	9	7	8
Sept. 5, 1994	7	18	9	12
Dec. 12, 1994	6	1	—	—
Jan. 19, 1995	40	42	50	46

[a]Sample taken on September 1, 1993, was representative of the overall experimental population at the start of the experiment.

Fecundity analysis

Definitions

Potential fecundity (F_P), defined as the number of vitellogenic oocytes in the ovary prior to spawning, was calculated using the gravimetric method (Bagenal 1978), i.e., $(W_o/W_S) \cdot N$, where W_o is the weight of the preserved ovary, W_S is the weight of the fixed subsample taken for counting, and N denotes the number of vitellogenic oocytes in the subsample. The term W_o/W_S is defined as the raising factor. Realized fecundity (F_R), defined as the number of eggs (fully developed cells) spawned in the current season, was calculated as $F_R =$

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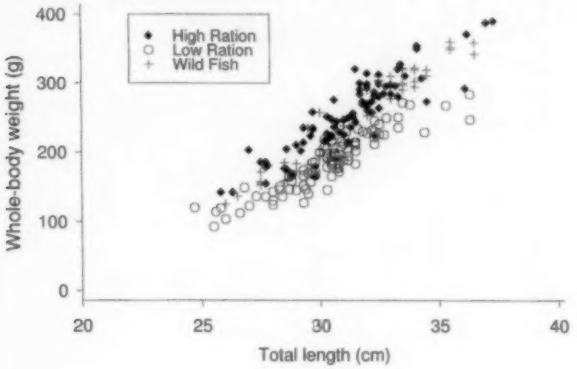
Fig. 3. Relationship between length and weight of the experimental fish at the end of the experiment (January 19, 1995). Also shown are data for wild fish caught in early February 1996 in the same area where the experimental fish were taken.



$F_P - N_{res}$, where N_{res}, the number of oocytes resorbed due to atresia, is given by

(2) $$N_{res} = F_P \cdot A \cdot D/T$$

where A is the relative atretic intensity, T is the turnover rate (i.e., life span in days of an early α-stage oocyte), and D is the estimated number of days from the sampling date until the assumed final (maximum) oocyte diameter is reached. Relative potential fecundity (RF_P) and relative realized fecundity (RF_R) were defined as $F_P/(W - W_g)$ and $F_R/(W - W_g)$, respectively.

Estimations of F_P and F_R

Estimates of F_P were made for fish in Hjort maturity stages 3–5 collected in January 1995. All estimated F_P values were finally corrected for cumulative atresia up to the expected time of spawning, thus indicating F_R. These calculations were all based on specimen-specific A and D values but a general T value. Variable A was assumed to be constant during the period from sampling until spawning for each individual fish (see Discussion).

To decide the minimum ovarian subsample weight needed to achieve stable counts prior to starting on the main F_P study, three subsamples of about 100, 150, and 200 mg, respectively, were taken from the middle part of the right ovary lobe of each of three specimens. Before weighing the ovaries, excess moisture was removed using absorbent paper. The weights of the preserved ovaries and the subsamples were recorded to the nearest milligram. Counts were made separately for each subsample size. The results showed no consistent trend and only minor differences (<2%) between the highest and lowest F_P recorded within a fish. Therefore, in the main study, a conservative subsample weight of 150 mg was used and two subsamples were taken from each fish at the middle part of the right lobe.

The turnover rate T in eq. 2 was set at 10 days assuming an experimental water temperature of 8°C. This value was based on the following estimates: 7–9 days (16°C) in northern anchovy (*Engraulis mordax*) (Hunter and Macewicz 1985), 10–13 days (8°C) in Atlantic cod (Kjesbu et al. 1991), and 7.5 days (temperature not given) in Atlantic mackerel (*Scomber scombrus*) (Anonymous 1993).

The variable D in eq. 2 was deduced from the following oocyte growth model. By assuming a final oocyte size (i.e., at commencement of spawning) of 1200 µm for the local Atlantic herring, a value found to vary little among years (A.-L. Agnalt, Institute of Marine Research, Bergen, Norway, unpublished data), the number of days it will take before a fish spawns can be estimated using the exponential equation

(3) $$Y = Y_{min} + ae^{bX}$$

where Y is mean oocyte diameter, Y_{min} is the minimum developing oocyte diameter, X is the number of days (starting from May 1 to the sampling day), and a and b are constants. Both diameters (micrometres) refer to fixed values in whole-mount preparations.

The available time series of diameter measurements were used in the model. Assuming the oocyte development rate being consistent between years, the diameter measurements reported in February 1994 were used to represent the same period in 1995. The model parameters were estimated using the nonlinear regression function in S-PLUS (Venables and Ripley 1994).

Results

Condition of experimental versus wild Atlantic herring

The use of ANCOVA on fish weight–length relationships at the end of the experiment (Fig. 3) demonstrated no significant differences in slope ($P = 0.08$) but in intercept ($P < 0.001$) (log–log transformed data). The latter result was due to the low-ration fish; the high-ration group was not significantly different (Tukey, pairwise comparative probability, $P = 0.08$) from the wild fish, while weight-at-length for the low-ration group was markedly lower ($P < 0.001$). The variance in weight within a length-class was large for both groups, but only a few fish of the high-ration group had weight-at-length similar to that of the low-ration group.

Seasonal and temporal changes in fish length, weight, GSI, and HSI

A significant overall increase in length during the experimental period was observed for all but the low-ration females ($P = 0.02$ and 0.008 for high-ration females and males, respectively, and 0.007 for low-ration males) (Figs. 4*a* and 4*b*). The estimated overall growth increment during the experimental period was 2–2.5 cm. Only at the end of the experiment was there evidence (t-test, $P = 0.02$) of a ration effect on mean length, and only for males.

The time series of whole-body and somatic weight (Figs. 4*c*–4*f*) showed indications of a seasonal cycle for the high-ration group. Females of the low-ration group showed a similar trend to that of the high-ration group for the first spawning season in captivity, although the weight loss was larger. However, contrary to the high-ration group, they did not regain this weight loss and from May 1994, there was evidence (t-test, $P < 0.05$) for a lower weight (both somatic and whole-body weight) of the low-ration group than that of the high-ration group. For the low-ration males the somatic and whole-body weight decreased marginally and no seasonal pattern was found.

There was evidence for a difference in GSI between the two ration groups of females at the last sampling date (t-test, $P = 0.04$) but not elsewhere ($P > 0.20$) (Fig. 4*g*). For males the difference in GSI was significant in both February 1994 ($P = 0.004$) and January 1995 ($P = 0.001$) (Fig. 4*h*). The data indicated that the seasonal maximum for males is reached later for the low-ration than for the high-ration fish.

Females of both groups had higher HSI than males (t-test, $P < 0.05$), except at the start of the experiment and in May 1994 (Figs. 4*i* and 4*j*). There were indications of a seasonal cycle in HSI for females. For males, this pattern was less clear (low-ration group) or nonexistent (high-ration group).

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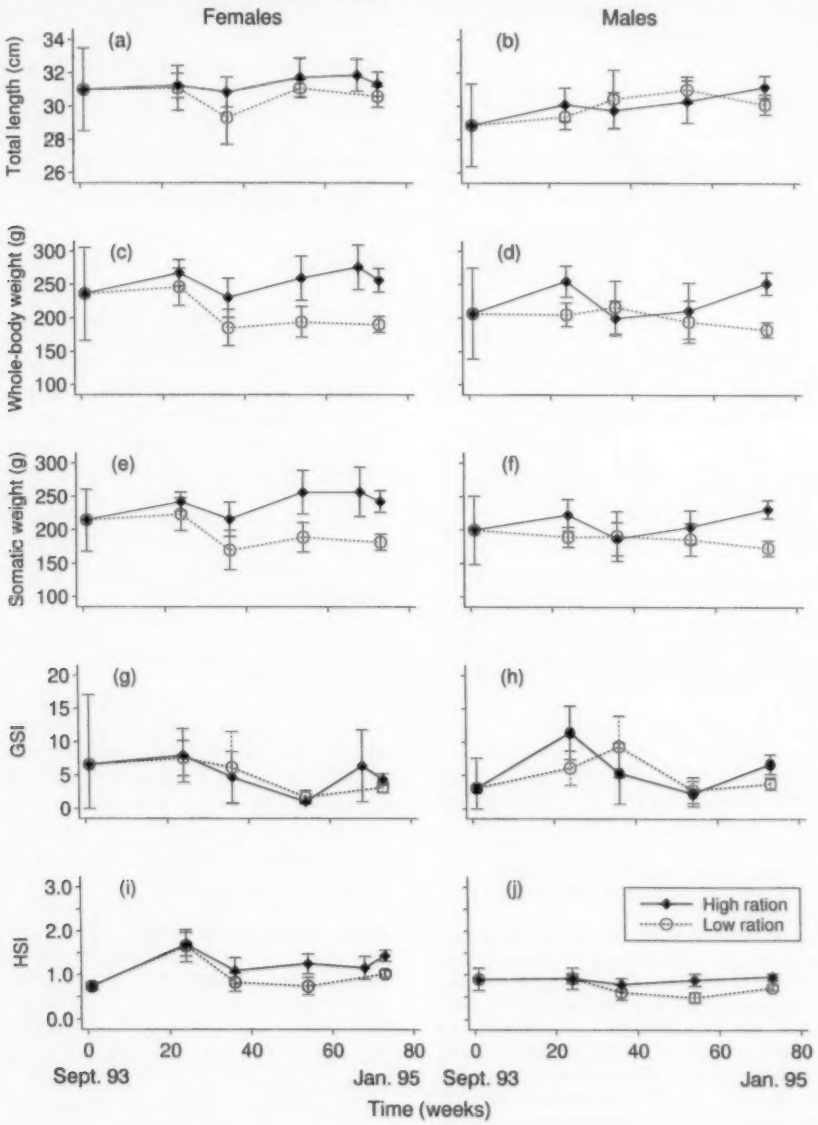
Fig. 4. Temporal changes in total length, whole-body and somatic weight, GSI, and HSI of the experimental fish and a comparison between the two ration groups and sexes. Mean values and 95% confidence limits are given.



Evidence for a ration effect on the values of the HSI was found for both sexes in the September 1994 (females: $P = 0.005$; males: $P < 0.001$) and January 1995 samples (females: $P < 0.001$; males: $P < 0.001$), i.e., after the first spawning season.

Oocyte diameter

Whole-mount diameter measurements under the binocular microscope (Y_{bino}) were closely correlated with those reported histologically with the image analyzer (Y_{image}):

(4) $Y_{bino} = -4 + 1.090 \cdot Y_{image}$ ($n = 49$, $r^2 = 0.984$, $P < 0.001$, $180 < Y_{image} < 1150$ µm).

The process of dehydration, embedding, and sectioning on average resulted in a total shrinkage of 7%. There were no indications of any oocyte size-specific differences in this degree of shrinkage.

As maturation advanced, i.e., vitellogenic oocytes examined under the binocular microscope grew in mean diameter from about 375 to 925 µm, the gap between the size–frequency distributions of vitellogenic oocytes and previtellogenic oocytes gradually increased from about 100 to 550 µm. For fish with mean vitellogenic oocyte diameter above 550 µm, the maximum previtellogenic oocyte diameter remained stable at 240 µm. In the less advanced maturity stages (mean vitellogenic oocyte diameter from 375 to 550 µm), it was estimated at 250 µm. From histological sections the maximum

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Fig. 5. Comparison of mean vitellogenic oocyte diameter at whole-body weight between the first and second year in captivity for the two ration groups.

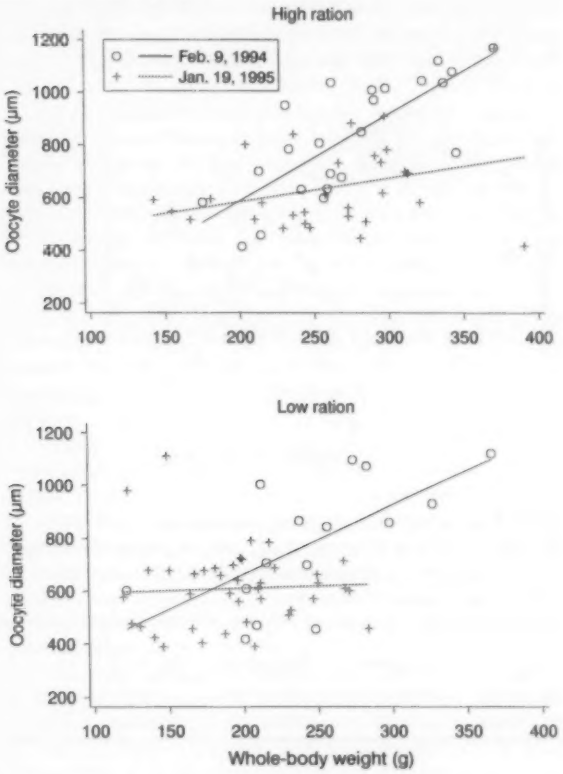
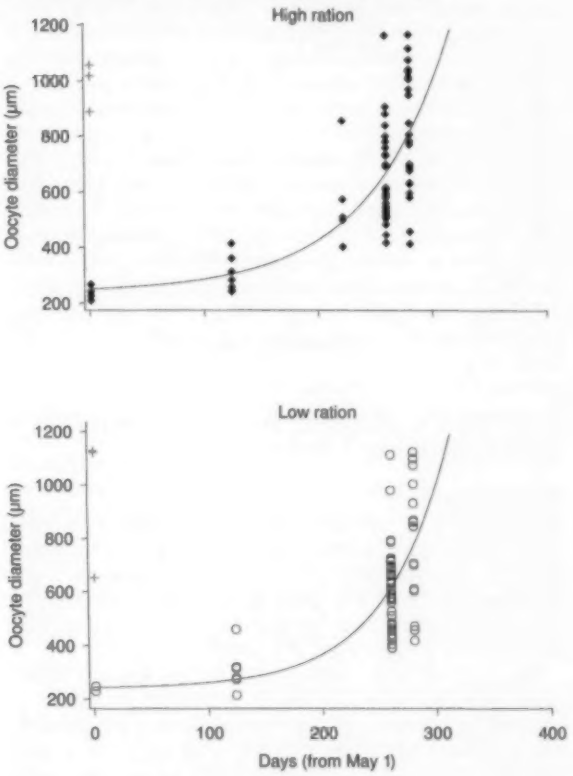


Fig. 6. Oocyte growth curve for the two ration groups. Fish excluded from the analysis are indicated by plus signs.



previtellogenic oocyte diameter was estimated at 230 µm after correcting for shrinkage using eq. 4. Thus, oocytes above 240 µm were considered developing.

There was no strong evidence for a relationship between whole-body weight or total length and mean vitellogenic oocyte diameter based on the data collected in January 1995, either for the high- or the low-ration group (Fig. 5). Omission of extreme values did not change this conclusion. Moreover, there was no evidence for a difference in oocyte diameter of the two ration groups. However, by studying the data collected 1 year earlier, in February 1994, oocyte diameter was found to increase with increasing weight for both groups (high ration: $r^2 = 0.59$, $P < 0.001$; low ration: $r^2 = 0.43$, $P < 0.01$) and also with increasing total length for the high-ration group ($r^2 = 0.34$, $P < 0.01$).

Oocyte growth pattern

Using a minimum developing oocyte diameter of 240 µm, the estimated growth curves were

$$(5) \quad Y = 240 + 11.59 \cdot e^{0.014x}$$

for the high-ration group and

$$(6) \quad Y = 240 + 3.67 \cdot e^{0.018x}$$

for the low-ration group (Fig. 6). The variation in oocyte diameter was very large in May (i.e., at the starting point) due to the mixture of spent and spawning fish. For the purpose of studying the developing oocyte growth pattern, the maturing and spawning fish found in this sample were excluded. Nevertheless, the among-fish variation in oocyte diameter was considerable. However, the estimated time needed to reach the assumed final oocyte diameter of 1200 µm was found to be very similar for the high- and low-ration females: 318 and 314 days, respectively.

Level of atresia

ANCOVA showed relative atretic intensity to increase with total length ($P = 0.007$) and whole-body weight ($P = 0.007$) (Fig. 7), but there was no evidence for a difference in slopes between groups ($P = 0.582$ for length as covariate, $P = 0.237$ for weight as covariate). The analysis further showed evidence for an overall higher level of atresia in the low-ration than in the high-ration group ($P = 0.021$ with length as covariate, $P = 0.003$ for weight as covariate). The above conclusions were not changed by excluding the notable outlier for the low-ration group. The average relative atretic intensities for the high- and low-ration groups were 3.0 and 5.7%, respectively. No evidence for a relationship between oocyte diameter and relative atretic intensity existed based on separate correlation analyses of fish from the high-ration group ($P = 0.581$) and the low-ration group ($P = 0.389$). Inclusion of fish condition measured

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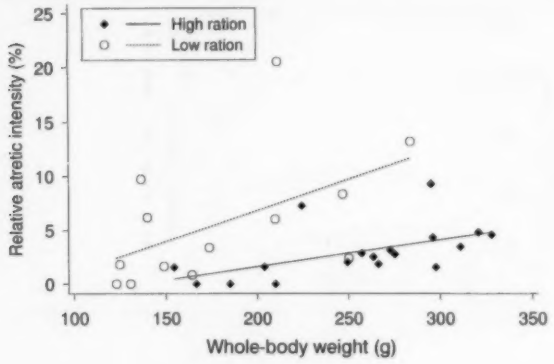
Fig. 7. Relationship between whole-body weight and relative atretic intensity for the two ration groups.



as SCF failed to significantly improve the understanding of within-group variation in atresia.

Fecundity analysis

Within each of the two ration groups, F_P and RF_P were found to be independent of whole-body weight (Fig. 8), total length, and SCF. The experiment provided strong evidence for higher F_P in the high- versus low-ration group (t-test, $P = 0.002$) (Table 2). However, RF_P was not found to differ significantly between the two groups (t-test, $P = 0.940$).

The F_R (i.e., after correction for atresia) was not significantly correlated with total length or whole-body weight for either of the two ration groups. High-ration fish had higher F_R than low-ration fish (t-test, $P < 0.001$), but there was no clear statistical evidence for a difference in RF_R between the groups (t-test, $P = 0.212$), although the low-ration group had a lower average value than the high-ration group (Table 2). The F_R was significantly lower (40% reduction) than F_P for fish in the low-ration group ($P < 0.01$), but no clear difference was found for the fish in the high-ration group (9% reduction). For the two low-ration fish with the highest relative atretic intensities (13.2 and 20.5%) the calculated F_R was zero (negative), i.e., all vitellogenic oocytes were likely resorbed before spawning. Both fish were in extremely poor condition.

Discussion

Two phenomena likely attributable to the impoundment and feeding regime were discernible. First, compared with the wild situation, the present way of feeding obviously reduced within-year variation in accumulated body reserves; wild Atlantic herring store significant amounts of body reserves (lipids) in the summer peak feeding period for subsequent use during winter fast (Henderson and Almatar 1989). Nevertheless, for the high-ration group, weight-at-length in January was comparable with that of wild fish across the whole length range. Second, a significant relationship between vitellogenic oocyte size and fish size was found based on the data collected in February 1994, i.e., after about half a year in captivity. However, at a similar stage in the following maturation cycle, no such relationship was found. Oocyte diameter is generally found to increase with fish size, and large fish spawn earlier than smaller ones (Lambert 1987; Ware and Tanasichuk 1989). It is

Fig. 8. Relationship between whole-body weight (W) and potential fecundity (F_P) for the two ration groups and wild Atlantic herring caught in the same geographical area as the experimental fish (A.-L. Agnalt, Institute of Marine Research, Bergen, Norway, unpublished data). The linear regression between F_P and W for the combined high-ration and wild Atlantic herring data was $F_P = 19\,046 + 267.7 \cdot W$ ($r^2 = 0.415$, $P < 0.001$).

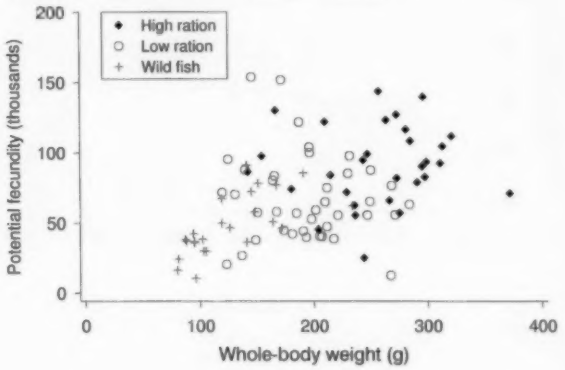


Table 2. Mean and standard error (in parentheses) of potential fecundity (F_P), realized fecundity (F_R), relative potential fecundity (RF_P), and relative realized fecundity (RF_R) for the two ration groups in the January 1995 sample.

Group	F_P (thousands)	F_R (thousands)	RF_P (g^{-1})	RF_R (g^{-1})
High ration	91.2 (5.2)	83.0 (7.8)	373 (27)	352 (52)
Low ration	67.2 (5.0)	40.4 (7.9)	369 (34)	254 (55)

suggested that this trend in wild fish is caused by fish of different sizes experiencing slightly different environmental conditions (e.g., temperature). Therefore, they mature at different rates (Kjesbu 1994). However, under identical environmental conditions, the fish size effect on oocyte size should become undetectable, as noted.

Lasting for 1.5 years, this study clearly demonstrated that Atlantic herring can maintain a normal development on an average diet of 12 mg wet weight·g $fish^{-1}$·day^{-1} (mean whole-body weight of high-ration fish was about 250 g and food intake was 3 g·$fish^{-1}$·day^{-1}). In contrast with other studies, this experiment covered the full maturation cycle. The estimate is lower than the indirect maintenance ration of 17 mg·g $fish^{-1}$·day^{-1} for Pacific herring calculated by Hay et al. (1988) and comparable with the lower range of the maintenance estimates (9.1–28.6 mg·g $fish^{-1}$·day^{-1}) for Atlantic herring found by Blaxter and Holliday (1963). However, estimates of required food rations based on controlled laboratory experiments may not fully apply to natural conditions, especially as wild Atlantic herring tend to undertake annual migrations which would increase their metabolic requirements. Actual food composition and ambient temperature may also be different. The present feeding regime of 5 days per week instead of 7 days per week is believed to have had minor impact. The gastric evacuation rate for krill is in the order of about 2 days at the ambient temperature, extrapolated from data on copepods assuming an evacuation constant for krill half that of

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copepods (Temming 1995; A. Temming, personal communication). Thus, our Atlantic herring did not likely suffer from periodic starvation. Based on the comparison of the length–weight relationship between the experimental fish and wild fish, the present estimate is assumed to give a realistic value of the required food requirements of wild Atlantic herring.

The low-ration group showed clear signs of inadequate food. For females belonging to this group, the fall in somatic weight following the first spawning in captivity was larger than for the high-ration females. Also, the ration fed was apparently insufficient to subsequently restore their somatic energy deposits depleted for reproductive investment. The low-ration males showed no seasonal pattern in somatic weight at all, but an overall weak decline. Investment in reproduction is generally larger for females than for males of the same size (Wootton 1990). This may explain why low-ration females were the only group to show no overall change in mean length. The temporal pattern of HSI supports these conclusions.

Presented female data on GSI, oocyte growth, and final mean vitellogenic oocyte size indicated no significant effect of ration size on time of spawning. GSIs for males indicated, however, delayed spawning for males within the low-ration group. During the late maturity stages (February 1994), GSI of males was significantly higher than that of females. Testes growth precedes ovary growth and males mature earlier than females (Iles 1964; Ware and Tanasichuk 1989). In contrast with our observations, Gillis et al. (1990) found that the mean GSI values for males were always lower than those for females from the time of population ovulation until the end of the spawning season. This might indicate that the maximum GSI for females may be underestimated. As pointed out by Gillis et al. (1990), GSI increases rapidly during ovulation, and more frequent sampling would have been needed to determine the GSI pattern at this time. For spring spawners, oocyte development, starting somewhere between May and September (Bowers and Holliday 1961; Iles 1964; Ware and Tanasichuk 1989), has been found to slow down or halt during part of the winter (Iles 1964). Thus, there might be a plateau somewhere on the oocyte growth curve. The length of the overwinter period differs among stocks. Besides, herring have been shown to be able to hold off spawning for several days, perhaps awaiting environmental cues (Iles 1964; Ware and Tanasichuk 1989). Thus, the present oocyte growth curve might fail to determine the exact spawning time. Despite this, it still should serve the purpose of a comparison of the two ration groups. Considering the large among-fish variation in oocyte diameter, the difference in estimated time at spawning is marginal.

The low-ration fish had a significantly lower F_P than the high-ration fish. RF_P was not different, which can be explained by the much lower somatic weight of the low-ration fish. It thus appears that the number of developing oocytes is determined by the nutritional status of the female. This statement is confirmed for a wide range of fish species (Bromage et al. 1991; Karlsen et al. 1995). Food restriction might also produce greater stress on the fish through increased competition, and this stress may lead to lower fecundity through changes in endocrine activity (Bagenal 1969). The F_P was unrelated to fish size in both ration groups. However, when similar data from samples of wild fish collected in the same geographical area (Fig. 8) were included in the analysis, this variable was significantly related to whole-body weight. Thus, given the large variation in potential fecundity-at-size, a larger contrast in size was needed to show the relationship that is clearly found in wild specimens (Almatar and Bailey 1989).

The histological analysis of the data collected at the end of the experiment indicated that atresia was a common phenomenon in these Atlantic herring. The low-ration fish had on average twice as high relative intensity (5.7%) of atresia as the high-ration fish (3.0%). The variation within each group was large, especially within the low-ration group. The significantly higher mean value in the low-ration fish is in agreement with other studies showing a progressive increase in atresia as the ration decreases below an optimal level (Bagenal 1969; Tyler et al. 1990; Kjesbu et al. 1991). An increase in atresia with fish size was also noted in both ration groups. The reason for this is unclear. When calculating F_R, relative intensity of atresia from the time of sampling until final maturation was assumed constant and the turnover rate set at 10 days. No relevant detailed study of the variation in relative intensity of atresia throughout the maturation cycle is known to the authors. However, no indications for a significant relationship between maturity stage (oocyte diameter), condition, and relative atretic intensity were found based on the individuals sampled in January 1995, even though these showed a wide range of oocyte sizes. Thus, the assumption of a constant relative intensity within the period of interest seems warranted but, obviously, further studies are needed on this topic as well as determination of the atretic turnover rate for Atlantic herring.

For the high-ration group, estimated mean F_R was 9% lower than the similar F_P as estimated at the end of the experiment. Due to the large variation in both potential fecundity-at-size and relative atretic intensity, the reduction was not significant. The reduction for the low-ration group was as high as 40%. The difference between RF_P and RF_R for the low-ration group indicated that the low-ration fish had to get energy by resorbing some vitellogenic oocytes to maintain normal development of the remaining oocytes. Although atresia was not an extensive phenomenon in their study, Hay and Brett (1988) observed a 20% reduction in mean vitellogenic counts during a 3-month period prior to spawning.

In summary, this study suggests a maturation strategy whereby Atlantic herring at the beginning of the maturation cycle start out with a number of developing oocytes that is in excess of what the fish can bring to full maturation and the fish will later resorb some vitellogenic oocytes and likely reallocate a part of this energy to other growing oocytes. At the group level the relative intensity of atresia varies negatively with food ration, but due to large variance, any negative effect of condition could not be shown statistically at the individual level. Size and growth rate of developing oocytes do not seem to be significantly affected by the nutritional status of the fish. Further laboratory studies are needed to determine the effect of within-year changes in ration size, particularly during the summer-recovering, peak feeding period, on energy allocation and potential fecundity coupled with a detailed monitoring of temporal variation in relative atretic intensity.

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Quantifying the uptake and release of cadmium and copper by the opossum shrimp *Mysis relicta* preying upon the cladoceran *Daphnia magna* using stable isotope tracers

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Abstract: *Daphnia magna* were uniformly labeled with the stable isotope tracers ^{113}Cd and ^{65}Cu and fed to the invertebrate predator *Mysis relicta* to determine the levels of release, assimilation, accumulation, egestion, and excretion of Cd and Cu when consumed in food alone. Samples were analyzed using ICP-MS and data analyzed using a mass balance model. The sloppy feeding behaviour of mysids resulted in the release of metals from the prey *Daphnia* in particulate and soluble forms. Mysids have been observed to consume selectively the soft-body portion of *Daphnia*, resulting in the discarding of particulates, mainly exoskeleton, while eating. The trade-off of a higher handling time is a highly assimilable diet. Assimilation efficiencies of the two metals (72% for Cd and 21% for Cu) were considerably higher than previously published values for *M. relicta*, likely due to a previous overestimation of metal egestion. Excretion appears to be an important mechanism by which mysids rid their body of assimilated metals, resulting in the further transfer of metals into the readily available dissolved phase. After a 94-h depuration period, 47% of ingested Cd and 53% of ingested Cu remained associated with the mysids in either the gut, body, or exoskeleton.

Résumé : Nous avons uniformément marqué des spécimens de *Daphnia magna* avec les isotopes stables ^{113}Cd et ^{65}Cu et nous les avons donnés comme nourriture à des spécimens de *Mysis relicta*, un invertébré prédateur, afin de mesurer la libération, l'assimilation, l'accumulation, l'expulsion et l'excrétion du Cd et du Cu d'origine exclusivement alimentaire. Nous avons soumis des échantillons à la spectrométrie de masse ICP et analysé les résultats au moyen d'un modèle de bilan massique. *Mysis* ne mangeant pas sa proie en entier, une partie des deux métaux s'est libérée sous forme soluble ou en particules. Nous avons en effet constaté que *Mysis* ne consomme que les parties molles de *Daphnia*, rejetant des particules de tissus, essentiellement d'exosquelette. Ce mode d'alimentation, plus lent, n'est pas sans avantage : la nourriture consommée est très assimilable. L'assimilation des deux métaux (72 % pour le Cd et 21 % pour le Cu) s'est révélée beaucoup plus importante que ne l'indiquaient jusque-là les publications pour *M. relicta*, vraisemblablement en raison de la surestimation de l'expulsion des métaux chez cette espèce. L'excrétion semble jouer un rôle important chez les mysidacés pour l'élimination des métaux assimilés, ce qui est une autre source de métaux en solution facilement accessibles. Après une période de dépuration de 94 h, une partie des deux métaux ingérés, soit 47 % du Cd et 53 % du Cu, subsistait chez les *Mysis*, dans le tube digestif, dans le reste du corps ou dans l'exosquelette.
[Traduit par la rédaction]

Introduction

The freshwater opossum shrimp *Mysis relicta* is an opportunistic omnivore, ingesting sediment and benthos by day and consuming phytoplankton and zooplankton (selectively feeding on cladocerans when available) during their diurnal migration into the surface waters at night (Lasenby and Langford 1973; Bowers and Grossnickle 1978; Cooper and Goldman 1980; Lasenby and Fürst 1981). In lakes where mysids are endemic, they form a large proportion of the diet of a number of freshwater fish species (Lasenby et al. 1986). With the intention of enhancing various freshwater sport fisheries, mysids have also been introduced into over 100 lakes where they have mainly had deleterious effects on target pelagic fish species (as an effective competitor for plankton, see Spencer et al. 1991; Lasenby 1991), yet have been found in the stomach contents of more benthic species such as brown trout (*Salmo trutta*), lake trout (*Salvelinus namaycush*), and burbot (*Lota lota*) (Lasenby et al. 1986).

Mysids have been shown to play an important role in the transport and recycling of contaminants (Van Duyn-Henderson and Lasenby 1986; Rasmussen et al. 1990; Northcote 1991; Cabana et al. 1994). Traditionally, sediments are assumed to be the ultimate sink for contaminants. However, the migratory habits of *M. relicta*, coupled with their omnivorous and varied diet and short gut-passage time, may be a route of contaminant cycling between the benthic and pelagic systems.

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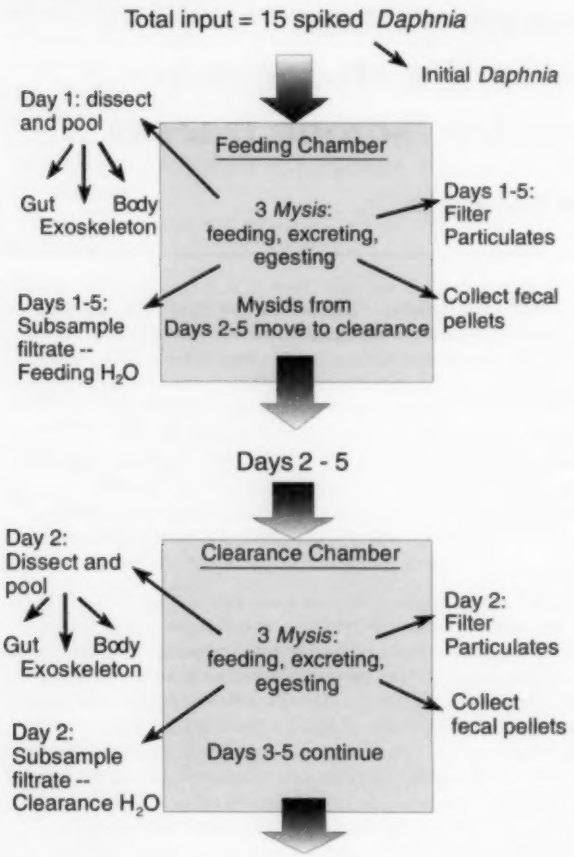
Fig. 1. Flow-chart of the experimental method showing all components of the experiment.



A mysid could potentially accumulate contaminants directly from the water, from contact with or consumption of the sediment, and from ingested food (Van Duyn-Henderson and Lasenby 1986; Val Klump et al. 1991; Lester and McIntosh 1994). Specific studies on the fate of metals taken up by mysids through the ingestion of zooplankton and sediments have concluded that only a small fraction of a metal is assimilated. The majority (>99%) is recycled to the coprophagous and decomposer community through sinking exuviae and fecal pellets (Van Duyn-Henderson and Lasenby 1986; Lasenby and Van Duyn 1992). These studies did not consider the release of metals, both particulate and soluble, during feeding and the fate of these metals within the organisms.

If the metals persist in the mysid gut over a number of days, the possibility of metal transport, along with metals incorporated into tissue, to higher trophic levels during this time remains open. The impact of mysid feeding on metal recycling could be further enhanced by the possible release of bound metal to the dissolved or free ion phase during feeding or depuration.

The purpose of this experiment was to determine the fate of ingested Cd and Cu using a mass balance technique with *M. relicta*, the stable isotopes ^{65}Cu and ^{113}Cd as tracers, and the cladoceran *Daphnia magna* as prey. The use of stable isotope tracers allows for the determination of the source of the metals to the predator and the partitioning of particulate and soluble metal released during feeding. As well, we determined the incorporation of metal into exoskeleton, body tissues, and gut tissue and contents and the release through fecal pellets and excretion over a 5-day period.

Methods

Daphnia magna were cultured in filtered lake water (0.45-µm membrane) spiked with ^{65}Cu and ^{113}Cd tracer solutions and were fed ^{65}Cu- and ^{113}Cd-spiked *Chlorella* and *Selenestrum* to attempt to mimic natural uptake of metals.

Mysids were collected from Crystal Lake, Ontario (78°19′W, 44°45′N), and maintained at 8°C. Fifteen prerinsed spiked *Daphnia* were added, five at a time, to each of twenty 1-L feeding chambers, filled with 8°C filtered lake water (Fig. 1). At the same time, five subsamples of 15 *Daphnia* were removed for determination of the initial concentration of tracer in these animals. Each *Daphnia* subsample was rinsed in distilled deionized water (ddH_2O) and placed on preweighed, predried, acid-washed plastic film and dried to a constant weight.

Three adult mysids, averaging 14.25 ± 0.14 mm (±SE) in total length, were added to 15 of the feeding chambers and allowed to feed on the *Daphnia* for an average of 4 h (Fig. 1). The remaining five chambers served as controls for release of tracer to the feeding chambers from *Daphnia* in the absence of mysids. During this time, three subsamples of three control mysids were taken, rinsed in ddH_2O, and dissected into three components: the exoskeleton (peeled off with legs and antenna added), gut, and the remainder of the body (including body fluids). The three components from all three mysids were pooled to increase the total sample mass per subsample available for analysis. All samples were placed on preweighed, predried, acid-washed plastic film and dried to a constant weight.

After feeding, mysids from three feeding chambers were removed, rinsed in ddH_2O, and dissected, pooled, and dried in the same manner as the initial mysids. Fecal pellets from these chambers were collected and placed on individual preweighed, acid-washed membrane filters and dried to a constant weight. Fecal pellets from the remaining 12 feeding chambers were collected and pooled onto one filter paper. To monitor the depuration of metals in a tracer-free environment, mysids from the remaining 12 feeding chambers were removed and transferred to 1-L clearance chambers (Fig. 1). After 24 h, unspiked *Daphnia* were added to the clearance chambers as prey.

The number of *Daphnia* remaining alive or intact (whole but not swimming, 2% of total added *Daphnia*) in the feeding chambers was counted, and the water in the feeding chambers was filtered through preweighed, pre-acid-washed 0.45-µm-membrane filters to collect the particulates (*Daphnia* fragments and whole *Daphnia*) to determine the amount of tracer not consumed by the mysids. The five *Daphnia* control chambers were also filtered to determine the proportion of tracer in the feeding chambers due to release from the *Daphnia* themselves. A 10-mL subsample of the filtrate from each chamber was collected for tracer analysis and acidified to 1% with concentrated subboiled HNO_3.

Twenty-two hours after feeding, mysids were removed and dissected and fecal pellets were collected as above from three of the 12 remaining clearance chambers (Fig. 1). Water from these three clearance chambers was filtered, and a 10-mL subsample of the filtrate was collected and acidified. Fecal pellets from the remaining 12 chambers were removed and pooled onto one filter paper. The same method was repeated 46, 70, and 94 h after feeding.

All dried samples and filters were placed into acid-washed teflon chambers with 300 µL of subboiled, concentrated HNO_3 and heated at 120°C for 6 h. These samples were transferred to preweighed

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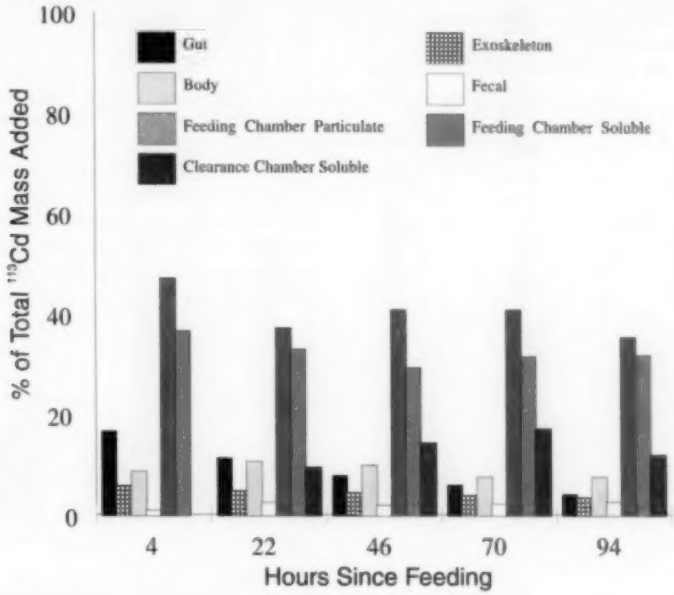
Fig 2. Percentage of total ^{113}Cd tracer added with initial *Daphnia* determined in all compartments over a 94-h period. Mass of tracer added with initial *Daphnia* = 25.6 ± 2.03 ng (mean ± SE).



15-mL polypropylene centrifuge tubes, diluted to ~6 mL, and reweighed to determine volume. To correct for metals present in the acid, filters, and plastic, a blank acid, a blank pre-acid-washed membrane filter, and a blank acid-washed plastic film were prepared and digested with each set of sample digestions. All samples were analyzed using ICP-MS. With each set of digestions, ~10 mg (weighed) of a standard reference material (oyster tissue, National Institute of Standards and Technology, standard reference material 1566*a*) was digested and analyzed for validation of the ICP-MS results.

Two isotopes of each metal were analyzed: (*i*) the tracer isotopes ^{65}Cu and ^{113}Cd and (*ii*) the nontracer isotopes ^{63}Cu and ^{114}Cd, which were used to correct for cross-contamination of tracer isotopes due to naturally occurring Cu and Cd. No correction for machine drift was found to be necessary and results were corrected for mass bias (Walder and Freedman 1992; Turner 1993; Walder et al. 1993).

The only source of tracer to the feeding chambers was from the addition of spiked *Daphnia*, and the only source of tracer to the clearance chambers was introduced with the mysids themselves. The tracer that was not ingested by the mysids would remain on the feeding filter or in the feeding water. The tracer ingested would be split among the mysid gut, exoskeleton, body, and fecal pellets. Since the only particulate source of tracer to the clearance chambers would be fecal pellets, tracer found on the clearance filters was assumed to be fecal pellets not collected with the pipette. The mass balance equation is as follows:

$$(1)\quad Mt_{Daphnia} = (Mt_{DP} + Mt_{Sol})_{FC} + (Mt_{Gut} + Mt_{Exo} + Mt_{Tissue} + Mt_{Fecal} + Mt_{Exc})_{CC}$$

where $Mt_{Daphnia}$ is the metal introduced with the initial *Daphnia*, Mt_{DP} is the metal associated with particulates in the feeding chamber, Mt_{Sol} is the soluble metal in the feeding chamber, Mt_{Gut} is the metal found in the mysid gut, Mt_{Exo} is the metal found associated with the mysid exoskeleton, Mt_{Tissue} is the metal associated with the remainder of the body, Mt_{Fecal} is the metal found in the mysid fecal pellets, and Mt_{Exc} is the soluble metal in the clearance chamber. The subscripts "FC" and "CC" represent the feeding chamber and clearance chamber, respectively.

Results

For both metals the observed concentrations of the standard reference material (3.88 ± 0.21 µg Cd/g and 60.19 ± 2.96 µg Cu/g, ±SD, n = 6 for both) were lower but within 10% of the certified concentrations (4.15 ± 0.38 µg Cd/g and 66.3 ± 4.3 µg Cu/g, ±SD).

An average of 25.60 ± 2.03 ng ^{113}Cd (±SE, n = 5) and 300.94 ± 27.38 ng ^{65}Cu (±SE, n = 5) was added to the feeding chambers through the addition of 15 spiked initial *Daphnia*. The mass balance calculation resulted in an average of 27.46 ± 3.49 ng Cd (107.3 ± 12.7% of total added tracer, n = 5, from day 1 to day 5, representing the sum of the mean daily mass of Cd from each component on the right side of eq. 1) and 341.10 ± 39.91 ng Cu (113.4 ± 11.7% of total added tracer, n = 5, as with Cd) recovered at the end of the experiment (Figs. 2 and 3). There was no significant difference between either the Cd or Cu added and recovered (independent t-tests: $t_8 = -1.056$, $p = 0.322$ for Cd and $t_8 = -1.695$, $p = 0.128$ for Cu).

For both metals, the majority of tracer remained in the feeding chambers in particulate and soluble form (Figs. 2 and 3). The particulate fraction can be further divided into mass as a result of live *Daphnia* and mass resulting from other particulates in the feeding chambers. Subtracting the average mass of tracer present as a result of *Daphnia* remaining whole at the end of feeding from the average mass of tracer in particulates remaining in the feeding chamber (Mt_{DP}) gives the mass of discarded *Daphnia* fragments. An average of 25.8% of Cd and 11.4% of Cu found on the feeding filters was associated with discarded particulates.

Results from the *Daphnia* control chambers indicate that 100% of Cd added with the initial *Daphnia* was recovered as particulates (independent t-test: $t_8 = 1.116$, $p = 0.297$), and no

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Fig. 3. Percentage of total ^{65}Cu tracer added with initial *Daphnia* determined in all compartments over a 94-h period. Mass of tracer added with initial *Daphnia* = 300.9 ± 27.38 ng (mean ± SE).

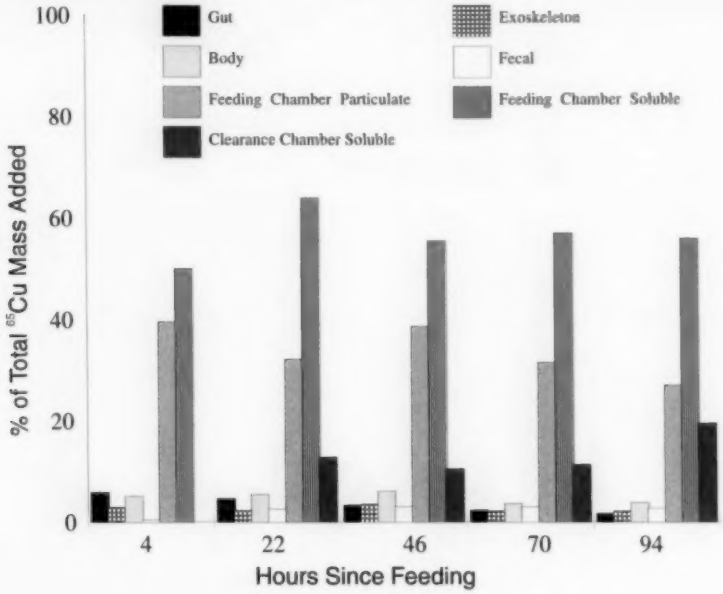
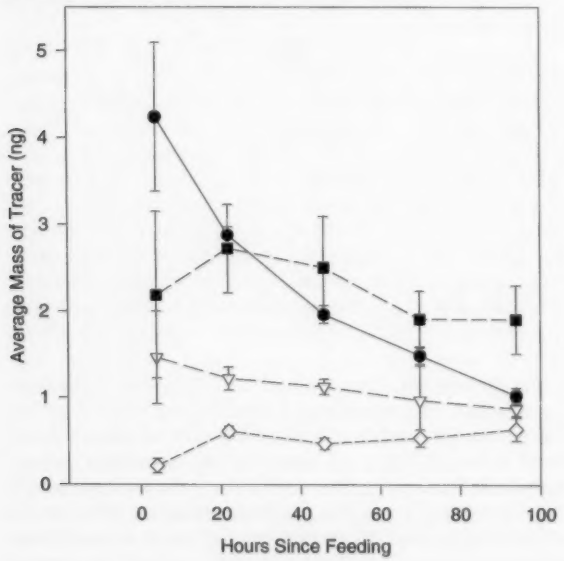


Fig. 4. Partitioning of ^{113}Cd tracer in *Mysis* gut (●), exoskeleton (▽), body (■), and fecal pellets (◇) over time after consumption of tracer-labeled *Daphnia* (mean ± SE). Cd measured after 4 h came from mysids from the feeding chambers; Cd measured at times 22–94 h came from mysids from the clearance chambers.



soluble Cd tracer was measured in the control water. For Cu, however, only 50% of total added tracer was recovered as particulates, resulting in 50% of the total added Cu released to the water in soluble form from the *Daphnia* themselves. In the feeding chambers with mysids, 56% of total added tracer was recovered in the feeding water. This increase in soluble Cu tracer in the feeding chambers may be a result of the release of tracer by mysids during feeding and (or) a change in the relationship between the release rate by *Daphnia* relative to the consumption rate of *Daphnia* by mysids.

The mass ingested by the mysids was calculated by summing the average mass of tracer measured in the gut (Mt_{Gut}), exoskeleton (Mt_{Exo}), body (Mt_{Tissue}), and fecal pellets (Mt_{Fecal}) 4 h after feeding. It was assumed that there was no excretion of tracer by the mysids over the 4-h feeding period. The metal tracer found in these mysids was considered representative of the mysids which were transferred to the clearance chambers. Of the total mass introduced with the initial *Daphnia* into the feeding chamber, 8.11 ± 1.82 ng Cd (31.7%, n = 3) and 41.66 ± 7.59 ng Cu (13.8%, n = 3) was ingested by the mysids and assumed to be transferred to the clearance chambers.

If the only source of tracer to the clearance chambers was that transferred with the mysids, a second mass balance formula for the clearance chambers is as follows:

$$(2) \quad Mt_{Mysis} = (Mt_{Gut} + Mt_{Exo} + Mt_{Tissue} + Mt_{Fecal} + Mt_{Exc})_{CC}$$

where Mt_{Mysis} is the metal introduced with the mysids to the clearance chamber, with the other parameters as defined for eq. 1.

On day 1, 4 h after feeding, about half (52%) of the ingested Cd (4.24 ± 1.45 ng, all subsequent n = 3) was found in the gut of the mysids, while about 27% of ingested Cd (2.19 ± 0.96 ng Cd) was already assimilated into the body of the mysids (Fig. 4). The mass in the gut steadily decreased to 13% of total ingested Cd (1.03 ± 0.15 ng Cd) 94 h after feeding on the spiked *Daphnia*, while the Cd mass in the body first increased 22 h after feeding (34%) and then decreased slightly by 94 h to 24% (1.91 ± 0.39 ng Cd). The mass of tracer found in the

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exoskeleton also followed a decreasing trend between 4 and 94 h after feeding (1.46 ± 0.54 to 0.88 ± 0.08 ng Cd, or 18 to 11%). Ninety-four hours after feeding, only 3.81 ± 0.43 ng Cd, about half (47%) of the ingested Cd tracer, remained in the mysids (sum gut, body, exoskeleton).

The movement of Cu from and within the mysid appears similar to the patterns observed with Cd tracer. The mass in the gut decreased steadily from 41% (17.13 ± 4.55 ng Cu) 4 h after feeding to 11% (4.65 ± 0.23 ng Cu) 94 h after feeding (Fig. 5). Initially the mass in the body increased, peaking at 43% (17.94 ± 6.54 ng Cu) 46 h after feeding, and then appeared to be leveling off 94 h after feeding at 27% (11.32 ± 2.72 ng Cu). As with Cd, about half (53%) of the ingested Cu tracer (21.92 ± 4.30 ng Cu) was measured as remaining in the mysids 94 h after feeding (sum gut, body, exoskeleton). A greater proportion of ingested Cu than Cd tracer was found in the fecal pellets (Figs. 4 and 5).

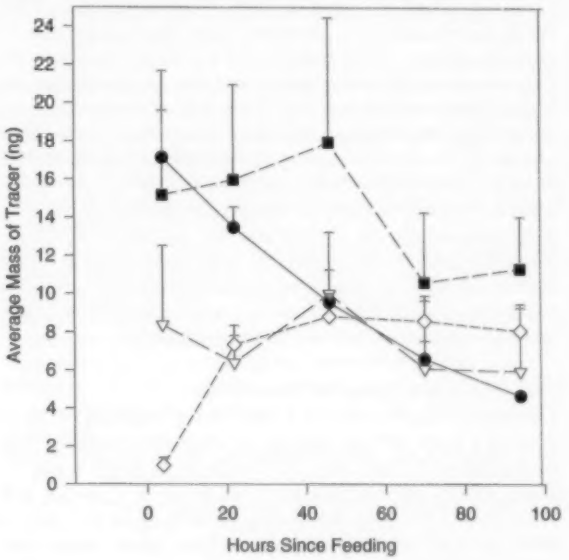
The assimilation efficiency for each metal 94 h after feeding was calculated as follows:

$$(3) \quad AE = [(I - F)/I] \times 100$$

where I is the mass ingested (nanograms; sum gut, body, exoskeleton, fecal, day 1) and F is the mass egested (nanograms; sum all fecal, days 2–5).

Mysid assimilation efficiencies were 72% for Cd and 21% for Cu. In other words, the mysids egested Cu 3.3 times more efficiently than Cd.

Fig. 5. Partitioning of ^{65}Cu tracer in *Mysis* gut (●), exoskeleton (▽), body (■), and fecal pellets (◇) over time after consumption of tracer-labeled *Daphnia* (mean ± SE). Cu measured after 4 h came from mysids from the feeding chambers; Cu measured at times 22–94 h came from mysids from the clearance chambers.



Discussion

Fate of metals not ingested by the mysids

The only source of tracer to this experiment was associated with the initial *Daphnia* introduced into the feeding chambers. When a *Daphnia* is attacked by a mysid, the metal associated with that *Daphnia* could be ingested by the mysid, remain associated with discarded *Daphnia* fragments, or be released to the water in soluble form. Most of the tracer mass introduced into the chambers was not ingested by the mysids and remained in the feeding chambers associated with live *Daphnia* and *Daphnia* fragments (particulate fraction) and in soluble (<0.45 μm) form (Figs. 2 and 3).

The *Daphnia* fragments associated with sloppy mysid feeding may also contribute to regeneration of metals within the water column. In marine studies, sinking copepod carcasses have been shown to release certain metals at an initially high rate greater than the release of carbon from carcasses (Lee and Fisher 1992). It was proposed that microbial activity has a large influence on the release of metals that penetrate into the cytoplasm of phytoplankton cells (e.g., Cd, Se, and Zn) and a negligible role in the release of particle reactive elements (e.g., Ag, Am, Ce, Pb, and Th) that associate with structural components of cells (Reinfelder and Fisher 1991; Lee and Fisher 1994). Release rates decreased exponentially over time, with the highest release rates found in the first 24 h of the experiment following the rates of C and protein release (Lee and Fisher 1993, cited in Lee and Fisher 1994). Although in shallower freshwater systems the majority of particles often reach the sediment within 24 h (Small and Fowler 1973; Van Duyn-Henderson and Lasenby 1986), it is possible that regeneration of at least a portion of the metals would occur in the surface waters of lakes.

Of particular interest is the large proportion of soluble tracer (8.28 ± 2.02 ng Cd (32%) and 169.32 ± 13.80 ng Cu (56%)) measured in the feeding water (Figs. 1 and 2). The *Daphnia* control chambers give some insight into the source of this soluble fraction. It appears that most of the soluble Cu found in the feeding chamber was released by *Daphnia* with only a minimal increase in the feeding chambers with mysids and *Daphnia*. These results are similar to those of Lee and Fisher (1994) who determined that as a result of zooplankton grazing on phytoplankton, the release of elements from particles into the dissolved fraction increased by 5–15%. Hutchins and Bruland (1994) found that biologically required trace metals (Fe, Zn, and Mn) behave in an analogous manner to major nutrients during grazing by zooplankton. Nutrients, such as N and P, are remineralized by grazers in surface waters which allows the reuse of these nutrients by primary producers. Results from this study support the findings of Hutchins and Bruland (1994) with the remineralizion of Cu, a biologically required trace metal, by zooplankton after grazing. However, the fact that no soluble Cd was found in the *Daphnia* control chambers suggests that the *Daphnia* were retaining Cd and that mysid predation had a significant impact on the release of this element into soluble form.

Without the use of stable isotope tracers, it would not have been possible to distinguish among the masses released by the mysids into soluble form and small particulate form and the contaminants already present in the water. These results support the hypothesis that mysids, as well as other raptoral predators with sloppy feeding habits, have a potentially greater impact on the recycling of metals in freshwater ecosystems

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than previously estimated, especially by the release of bound metal into soluble, readily available forms.

Due to the presence of soluble tracers in the feeding chambers, especially Cu, it is possible that some of the uptake of metals by the mysids was via the water and not through the ingestion of food (Glynn et al. 1992; Timmermans et al. 1992; Wang and Evans 1993). However, it has been shown that organisms deposit metals from ingested food in exoskelton tissues as a means of detoxification and storage (Reinfelder and Fisher 1994). Furthermore, the proportion of soluble metals in the feeding chambers was measured after 4 h of feeding time and therefore clearly overestimates the average exposure of the mysids during feeding. It was therefore assumed in this experiment that metals measured in the mysid postfeeding chamber were ingested by the mysids and not accumulated via the water. The addition of an alternate stable isotope tracer (in the case of Cd, there is no alternate stable isotope for Cu) to the water may allow the derivation of a relationship between dietary and aqueous sources of metals to organisms.

Fate of metals ingested by the mysids

The results indicate that 94 h after tracer exposure, 47% of ingested Cd and 53% of ingested Cu remained associated with the mysids in the gut, body, or exoskeleton (Figs. 4 and 5). These results are inconsistent with those of Lasenby and Van Duyn (1992) who found that 73–99% of the Cd and Zn taken up by *M. relicta* was egested in fecal pellets after a 12-h depuration period. This high egestion rate was attributed to the low assimilation efficiencies of metals (<20%), with the bulk of the metal passing through the organism unassimilated. Our results suggest that their egestion rate of metals was overestimated, likely as a result of the binding of metals to the peritrophic membrane of fecal pellets after egestion (Martin et al. 1990).

The method used to calculate assimilation efficiency is dependent on an accurate measurement of the egestion of the metals (eq. 3). It is possible that metals were scavenged out of the water by recently released fecal pellets; however, another factor confounding the measurement of egestion was the possible release of metals by fecal pellets after egestion (Fisher et al. 1991).

The mysid gut was easily dissected intact from the rest of the body and therefore included both the gut contents and the gut wall. It was surprising to find that even after 94 h, 12.7% of ingested ^{113}Cd and 11.2% of ingested ^{65}Cu remained in the gut; however, it is unclear whether this mass was in the gut contents or whether it remained associated with the gut wall. Because of the metals remaining in the gut, the assimilation efficiencies calculated using eq. 3 for both metals are only applicable 94 h after feeding and may be overestimated. However, despite the probability that the tracer metal burden in the mysid would lower with further excretion and egestion, it is clear that a high percentage of ingested metals remains available to mysid predators up to 1 week after ingestion.

Numerous studies have used radioactive tracers to monitor the flux of metals in fish after consumption of spiked food (Glynn et al. 1992; Harrison and Curtis 1992; Reinfelder and Fisher 1994). Although the use of a radioactive tracer should behave the same as a stable isotope tracer in reducing sample contamination, these studies found that most of the ingested metal passed through the fish via the fecal pellets. Glynn et al. (1992) found that minnows (*Phoxinus phoxinus*) retained only 5.6% of the ^{109}Cd ingested from spiked *D. magna*, and juvenile Atlantic (*Menidia menidia*) and inland silversides (*Menidia beryllina*) had an absorption efficiency of only 2.7% Cd when fed spiked copepods (Reinfelder and Fisher 1994). Reinfelder and Fisher (1994) hypothesized that the low uptake rates were a result of the high association of metals with exoskeleton of the zooplankton prey (97.4% Cd) whose chitinous carapace is difficult to digest. It was further determined that almost 80% of the elements associated with the soft (nonexoskeleton) tissues of copepods was available to be absorbed by juvenile planktivorous fish (Reinfelder and Fisher 1994).

Unlike many fish, which often consume the entire zooplankter, mysids selectively ingest soft tissues of their prey, discarding considerable exoskeletal debris while feeding (Lasenby and Fürst 1981; Figs. 2 and 3). The trade-off for the high initial handling time is a highly assimilable diet. In a radiotracer experiment using another invertebrate predator with sloppy feeding habits, *Bythotrephes cederstroemi*, it was found that 85% of C and P ingested with *Daphnia* was assimilated as a consequence of a fastidious feeding behaviour (Lehman 1993). Nonmetals, such as C and P used by Lehman (1993), have been found to have greater absorption efficiencies than trace metals in juvenile fish (Reinfelder and Fisher 1994). The high metal assimilation efficiencies found in this study, 72% for Cd and about 21% for Cu, could therefore be a consequence of the selective feeding behaviour of mysids.

Stephenson and Turner (1993) used radioactive ^{109}Cd to model the Cd dynamics within the grazing amphipod *Hyalella azteca*. Their study found an assimilation efficiency of 80% of Cd when ingested in natural periphyton. These authors admitted that although 80% is high compared with the literature, they were confident in their methods and concluded that the high assimilation efficiency is the result of coincidental uptake of Cd in the Ca-poor environment of their study lake. Crustacea have a very high metabolic Ca requirement for calcification of their exoskeleton, and Cd and Ca have similar ionic properties. With similar ionic properties, Cd and Ca enter organisms through the same type of channel or binding sites on the cell membrane without preference (Wang and Evans 1993).

The assimilation efficiencies of metals found in this study were considerably higher than those found for fish and slightly lower than those found for *Hyalella*. The crustacean *M. relicta* should have a similar high metabolic Ca requirement; however, as Ca was more readily available in this study (30–40 mg Ca/L (Ontario Ministry of the Environment 1976) versus 2–3 mg Ca/L (Stephenson and Turner 1993)), it should lower the possibility of Cd adsorption onto binding sites and result in a lower accumulation in the tissue (Wang and Evans 1993). Also, it is probable that some undigestible chitinous exoskeleton (Reinfelder and Fisher 1994) was ingested which would result in a lower assimilation efficiency for a mysid feeding on zooplankton than *Hyalella* feeding on highly digestible periphyton (Stephenson and Turner 1993).

In further support of this study, Hare et al. (1991) concluded that the primary source of ^{109}Cd and ^{65}Zn to the borrowing mayfly (*Hexagenia rigida*) was from uptake by the gut, and not via the gills from the surrounding water. Studies on the uptake dynamics of Cd and Zn in other invertebrate predators have concluded that the major path of uptake for Cd was

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through food whereas uptake of Zn occurred mainly through water (Timmermans et al. 1992). Differences in uptake were regarded as typical examples of the difference between essential (Zn) and nonessential (Cd) metals: uptake of Cd from both sources was large and rapid whereas increases in Zn concentrations were limited and seemed to be regulated (Hare et al. 1991; Timmermans et al. 1992).

In summary, we have shown that mysids feeding on zooplankton could have a dual effect on the recycling and transport of metals in the aquatic environment. Their sloppy feeding behaviour results in significant contributions of metals to the readily available soluble form and to the decomposer community. About half of ingested metals (47% Cd and 53% Cu) remain associated with the mysids even after a 94-h depuration period and are therefore available to higher carnivores. Excretion of metals was difficult to quantify; however, it appears to be a mechanism by which mysids rid their body of metals, further contributing to the dissolved pool of metals. The migratory habits of *M. relicta* spread the impact that this predator has on the recycling and transport of metals to both the benthic and pelagic zones of lakes that they inhabit.

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Marine reserves: patterns of adult movement of the coral trout (*Plectropomus leopardus* (Serranidae))

Dirk C. Zeller and Garry R. Russ

Abstract: Movements of *Plectropomus leopardus* (Serranidae), a major fisheries species, across marine reserve boundaries were investigated on the Great Barrier Reef, Australia. Mark–release–recapture and ultrasonic telemetry were used to assess movements. Mark–release–recapture used hook and line as the method of capture and underwater visual census (UVC) as the "recapture" tool. Catch rates were significantly higher in zones closed to fishing, despite UVC indicating no significant differences in density between closed and open zones. Of 183 fish marked with numerical freeze brands, 93 estimates of movements of branded fish were obtained. No branded fish was recorded to cross the reserve boundaries during the 2-month study, probably due to the initial decision to allocate capture effort evenly across the study area, rather than concentrating it on reserve boundaries. Fish carrying ultrasonic transmitters, and having home ranges straddling reserve boundaries, crossed boundaries on average 15.3 times·month^{-1}. The mean distance moved by freeze branded specimens between capture and recapture was significantly larger in areas closed to fishing than in those open to fishing. However, mean distance moved per day determined by ultrasonic telemetry did not differ between areas closed and open to fishing. This study suggests low flux rates of adult *P. leopardus* across marine reserve boundaries.

Résumé : Les déplacements de *Plectropomus leopardus* (Serranidae), une espèce faisant l'objet d'une importante exploitation commerciale, de part et d'autre des frontières d'une réserve marine ont été étudiés dans la région de la Grande Barrière, en Australie. Pour évaluer les déplacements, on a utilisé une méthode de marquage–recapture et la télémétrie ultrasonique. Pour le marquage–recapture, on a capturé les poissons à l'hameçon, et la « recapture » a consisté en un relevé visuel sous-marin. Les taux de capture étaient significativement plus élevés dans les zones où la pêche était interdite, alors que, selon les relevés visuels sous-marins, il n'y avait pas de différences significatives de densité entre les zones où la pêche était interdite et les zones où la pêche était permise. Sur 183 poissons marqués avec des cryomarques numériques, on a obtenu 93 estimations de déplacements de poissons marqués. Aucun poisson marqué observé n'a traversé les frontières de la réserve durant les 2 mois de l'étude, probablement à cause de notre décision initale de distribuer l'effort de capture uniformément dans l'aire d'étude plutôt que de le concentrer aux frontières de la réserve. Les poissons portant des émetteurs ultrasoniques et dont le domaine vital chevauche les frontières de la réserve ont traversé les frontières 15,3 fois·mois^{-1} en moyenne. La distance moyenne parcourue par les spécimens cryomarqués entre la capture et la recapture était significativement plus grande dans les zones où la pêche était interdite que dans les zones où la pêche était permise. Toutefois, les distances moyennes parcourues par jour, déterminées par la télémétrie ultrasonique, étaient similaires dans ces deux types de zone. Cette étude laisse penser que le flux de *P. leopardus* adultes aux frontières de la réserve marine est faible.
[Traduit par la Rédaction]

Introduction

Information about movement patterns of fish is central to the current debate about the use of marine reserves as a fisheries management tool for coral reef environments (e.g., Roberts and Polunin 1991; Russ and Alcala 1996*a*, 1996*b*). In a fisheries context, the two major objectives of marine reserves are to ensure continued recruitment supply via maintenance of a critical minimum spawning stock biomass and net export of larvae from reserves (the "recruitment" effect) and potentially to increase or maintain local fishing yields through export of adult biomass of target species from the protected areas to adjacent areas (the "spillover" effect) (e.g., Polacheck 1990; DeMartini 1993; Russ and Alcala 1996*a*). Marine reserves may form a more cost-effective management option for stock maintenance (e.g., Alcala and Russ 1990; Rowley 1994), particularly for coral reef fisheries, in which more conventional fisheries management strategies are especially difficult to administer (e.g., Russ 1991). The effectiveness of marine reserves, especially in the developing world, appears strongly linked to local fishing community support (e.g., White et al. 1994). Under these circumstances, suggestions of large spatial scale benefits (i.e., stock-wide recruitment) appear often less convincing to communities than promises of local benefits (i.e., spillover) (Russ and Alcala 1996*a*). Thus, demonstration of movements from protected areas, potentially leading to improved yields in areas adjacent to reserves, may be critical to the acceptance by local fishing communities of marine reserves as a fisheries management option. Therefore, empirical data on rates of movement and crossings of reserve boundaries are required.

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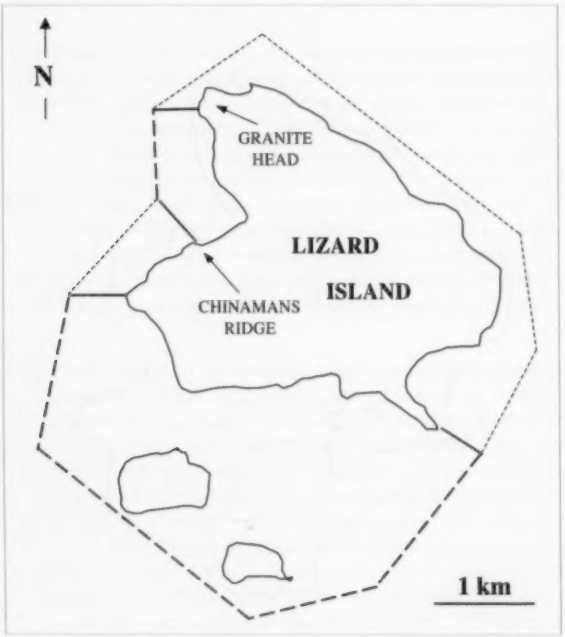
Fig. 1. Map of Lizard Island showing the general study area. Indicated are the Great Barrier Reef Marine Park management zone patterns and associated reserve boundaries. The study was limited to the north, northeastern, and western sides of the island, which were more sheltered from the prevailing southeast winds. The short-dashed lines outline the zones open to fishing for commercial and recreational fishers, the long-dashed lines outline the zones closed to fishing (reserves), the solid lines indicate the locations of marine reserve boundaries, and the dotted line is the reef contour outline.



The most common method to quantify movements of post-settlement fish is a mark–release–recapture study (e.g., Hilborn and Walters 1992) using external tags of the anchor or dart tag type (e.g., Recksiek et al. 1991). External tags are economical, small, and fast to apply to the animal and permit large samples to be tagged. However, tag loss rates are high in coral reef fishes (e.g., Whitelaw and Sainsbury 1986). An alternative tagging method uses thermal marking techniques. With fishes, both hot (e.g., Hargreaves 1992) and cold branding (e.g., Knight 1990) have been used. Retention times of marks are highly variable and species specific, with 2 years the maximum period recorded (Hargreaves 1992).

Tagged fish can be recaptured by the same fishing gear used for initial capture or be resighted by underwater visual census (UVC). The established suitability of UVC for assessing coral trout (*Plectropomus leopardus*) (Serranidae) populations and the satisfactory levels of readability of cold brands reported for coral trout (Samoilys 1987) provide an unique opportunity to utilise standardised underwater census techniques of individually identifiable fish to assess movement patterns of coral trout.

Ultrasonic telemetry is probably the most effective means of obtaining information on fish movements (e.g., Zeller 1997*a*, 1997*b*, 1998). The suitability of ultrasonic telemetry as a tool for fisheries research is only slowly being realised (Nelson 1990). Telemetry can make remote tracking of movement possible. Such an approach may overcome the shortcomings of traditional mark–release–recapture studies using external markings (Nelson 1990). Furthermore, it circumvents restrictions of visual observations on SCUBA, which limit most investigations of coral reef fishes to studies of smaller species in shallow water.

The primary objective of this study was to determine patterns of adult movement and space use by coral trout with respect to marine reserve boundaries. The initial predictive hypothesis was that flux rates are affected by potential density gradients in relation to marine reserve boundaries. The secondary objective was to compare two different techniques for assessment of movements: mark–release–recapture and ultrasonic telemetry. It was hypothesised that the two techniques should reveal similar patterns of movement. This is the first study to compare conventional mark–release–recapture (external marking combined with UVC) with ultrasonic telemetry on the same species in the same location.

Materials and methods

Study site and sampling protocol

The study was conducted on the sheltered western and northern sides of Lizard Island, northern Great Barrier Reef, Australia (14°40′S, 145°28′E). The study area contained within-reef marine park management zone boundaries between areas where fishing was and was not allowed (closure duration 12 years, Fig. 1). The external mark–release–recapture study was undertaken during August–October 1995, while ultrasonic tracking was conducted throughout 1993–1995.

Initial capture of specimens for marking was by hook and line, undertaken by two contracted commercial fishers in August 1995, each working independently from 4.1-m aluminium dinghies and each using standard commercial reef line fishery techniques. To distribute effort evenly, the study area was divided into four sections, each ~3 km long. Each fisher fished two "sessions" per day (morning and afternoon), with average duration per session of 4 h. Each location where a dinghy anchored and fished will be referred to as a "hang." To spread fishing effort evenly within the four sections, minimum (15 min) and maximum (60 min) hang times were allocated. Maximum fishing depth was set at 10 m to facilitate recaptures by visual census (see below). Coral trout caught at each hang were kept in bins supplied with fresh seawater. For each hang, fishers recorded location (using aerial photographs), start and finish time of fishing (hook-line hours), number of coral trout caught, and the storage bin corresponding to each hang. Hook-line hours were defined as the actual time period a baited hook was in the water and available to the fish. All incidental, nontarget bycatches were released. At the end of fishing a hang, the location was marked with a small, numbered marker buoy attached to a lead weight. This permitted cross-validation of location of individual hangs at the end of each session and allowed fishers to release marked fish at their exact capture location. Collected fish were brought to a centrally anchored boat after each hang for freeze branding.

Freeze branding technique

Prior to freeze branding, each fish was measured (fork length, FL) and tagged with two standard T-bar anchor tags (Hallprint Ltd., Holden Hill, Australia). Individual numerals were freeze branded onto the caudal peduncle on each side of the specimen using branding irons. Branding irons consisted of copper block bases with individual numerals (25 × 25 × 2 mm) on each iron. The branding duration for each numeral was 10 s, and fish were returned to holding tanks between

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each numeral brand to reduce stress. To ensure proper recooling of branding irons between applications, each numeral was returned to the coolant (liquid nitrogen, –196°C) for at least 1 min before reuse. After branding, individuals were released at the exact location of capture. In February 1995, a freeze branding trial showed that numerals remained readable underwater for a maximum of 3 months.

Resighting method and recapture effort distribution

"Recaptures" were resightings of freeze branded specimens during UVC. Two separate resighting surveys were made during September (D.C. Zeller) and October (D.C. Zeller and G.R. Russ) 1995, being 1 and 2 months after the freeze branding, respectively. Resighting effort, while spread over the complete study area, was concentrated in those areas with highest densities of freeze branded fish. General emphasis was placed on reef slope habitats, where coral trout are most abundant (Kingsford 1992).

The sampling unit was a 100 × 5 m transect. The transect length of 100 m was chosen to minimise the occurrence of zero counts of target species. Choice of transect width (5 m) was based on the minimum underwater visibility encountered around Lizard Island. Transect tapes were laid while conducting the surveys to avoid attraction of or disturbance to the fish by previously laid tapes (Fowler 1987). Start and end points of each transect were marked with surface buoys for recording the spatial position of transects from the surface after each census. All censuses were between datum (lowest astronomical tide) and the 10-m depth contour (or sand–reef interface), with an average census depth of 6–8 m. A total of 167 and 141 transects were sampled in September and October 1995, respectively, covering a total area of 154 000 m^2. During each census, all coral trout sighted within the transect width and up to 5 m in front of the projected transect line were counted and their location along the transect recorded to the nearest metre. Sightings of tagged fish were recorded and visible freeze brands identified. Any tagged coral trout observed outside the transect were investigated similarly and recorded, but clearly marked as a nontransect recapture on the data sheets.

Ultrasonic telemetry

Of 39 individual coral trout tracked using ultrasonic telemetry between 1993 and 1995 by Zeller (1997*a*, 1997*b*, 1998), eight fish had home ranges straddling existing management zone boundaries in the study area. The methods used for ultrasonic telemetry are described in Zeller (1997*a*, 1997*b*). The average tracking period per specimen for the eight fish straddling reserve boundaries was 52.3 days.

Data analysis

The spatial position data for hook and line capture locations, visual census transects, fish sighting locations, and ultrasonic tracking positions were digitised from calibrated aerial photographs. Distances moved between capture and resighting and during ultrasonic tracking were calculated using CALHOME© (Kie et al. 1996). Patterns of movement were evaluated with respect to reef habitat type (fringing versus patch reefs), management zones (open versus closed to fishing), reserve boundaries, and time period between recaptures:1 month between initial capture on hook and line (August 1995) to first UVC (September 1995, UVC 1), 2 months between initial capture to second UVC (October 1995, UVC 2), and 1 month between first and second UVCs (UVC 1–2).

Statistical analyses included *t*-test, analysis of variance (ANOVA), χ^2 homogeneity tests, and linear regression. Statistical assumptions underlying the parametric analyses were examined prior to analysis and data were $\log_{10}$ transformed where appropriate (Sokal and Rohlf 1981). All means are reported as means ± SE.

Results

Initial capture of coral trout on hook and line for freeze branding resulted in a fishing effort of 174 individual hangs consisting of 72 h and 5 min of hook and line fishing. Thus, on average, each hang was fished actively for 24 min. A total of 216 coral trout were caught, resulting in an overall catch-per-unit-effort (CPUE) of 2.9 coral trout·hook-line $hour^{-1}$. The CPUE differed significantly between management zones closed and open to fishing (*t*-test: $t_{[172]} = 3.07$, $p = 0.003$), with a CPUE of 3.2 ± 0.50 and 1.2 ± 0.26 coral trout·hook-line $hour^{-1}$ inside reserves ($n = 100$ hangs) and in open zones ($n = 74$ hangs), respectively. Of the 216 captured fish, 21 were judged to be injured and sacrificed, and 12 were kept for ultrasonic transmitter implantation (additional tracking was undertaken between 1993 and 1995, Zeller 1997*a*, 1997*b*). Hence, 183 of the initial 216 coral trout were released at their capture site after tagging and freeze branding.

The total available reef area, as defined for the visual census survey (datum to 10 m) comprised 750 966 m^2 in the study area (44% open, 56% closed to fishing). Given that the September (167 transects) and October (141 transects) surveys covered an area of 154 000 m^2, the two surveys sampled 11.1 and 9.4%, respectively, of the available, census-defined reef in the study area.

During the two UVC surveys, 817 coral trout were sighted, consisting of 440 and 377 fish for the first and second census, respectively. Of the total sightings, 44 (UVC 1) and 26 (UVC 2) were recaptures (resighting) of freeze branded individuals. Thus, recapture (resighting) rates were 24.0% (44 of 183) and 14.2% (26 of 183) for UVC 1 and UVC 2, respectively. Based on the total fish counts and area covered by the transects, an overall density estimate of 5.3 ± 0.26 coral trout·1000 m^2 reef $area^{-1}$ was derived. A breakdown of total census data by reef habitat (i.e., fringing versus patch reef) indicated counts of 362 and 455 coral trout in the 128 and 180 transects conducted in fringing and patch reef habitats, respectively. These counts resulted in density estimates of 5.7 ± 0.40 coral trout·1000 m^{-2} for fringing reef and 5.1 ± 0.34 coral trout·1000 m^{-2} for patch reef habitats. Evaluation of UVC data by reef management zones (i.e., open versus closed to fishing) showed that 164 and 653 coral trout were counted in the 72 and 236 transects sampled in the open and closed zones, respectively. Thus, density estimates of 4.6 ± 0.39 and 5.5 ± 0.32 coral trout·1000 m^{-2} were obtained for open and closed zones, respectively. Density estimates for open and closed zones did not differ significantly (*t*-test: $t_{[306]} = 0.49$, $p = 0.624$).

Distances moved

A total of 93 measures of distances moved by freeze branded specimens between time periods were recorded during the two visual census surveys (Table 1). The distance measures obtained represent linear distances between points of capture and (or) visual recapture. Multiple resightings (two or more sightings of the same indvidual) and resightings recorded outside the predetermined transect limits while censusing were included also. There was no clear relationship between distances moved and size of individual fish ($r^2 = 0.032$, $p = 0.086$, $n = 93$). The movement distance estimates obtained through resightings ranged from 4 to 387 m (overall mean = 94.9 ± 9.48 m) between individual points of capture and (or) resighting.

Mean distance moved between recapture occasions differed between the two reef habitats (fringing and patch reefs) examined (*t*-test: $t_{[91]} = 2.92$, $p = 0.004$). Fish on patch reefs moved,

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Table 1. Sample sizes of distances moved for coral trout between the three time periods and two-factor ANOVA comparing distances moved of freeze branded fish between the three time periods and between management zones.

Time period	No. of distances obtained: Open	No. of distances obtained: Closed	No. of fish with multiple sightings
UVC 1[a]	9	43	5
UVC 2	7	24	1
UVC 1–2	3	7	1
Total	19	74	7
Grand total		93	

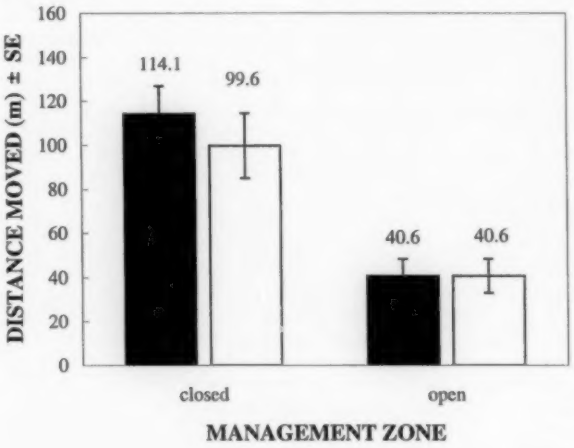
Source of variation	Mean square	df	*F*	*p*
Fringing and patch reef fish ($n = 93$)				
Time period	0.4019	2	2.308	0.1055
Zone	1.3266	1	7.617	0.0071
Time period × zone	0.1235	2	0.709	0.4949
Residual	0.1742	87		
Fringing reef fish only ($n = 56$)				
Time period	0.1735	2	0.824	0.4447
Zone	0.8995	1	4.269	0.0440
Time period × zone	0.1634	2	0.775	0.4659
Residual	0.2107	50		

[a]Example for UVC 1: 44 individual marked fish were resighted (see Results section), of which five fish were resighted two or more times (i.e., 39 single sightings, two double sightings, and three triple sightings), resulting in a total number of distances obtained of 52 (i.e., nine in open plus 43 in closed zone).

on average, 48.3 m more between recapture occasions than fish on fringing reefs (patch reefs: mean = 129.0 ± 20.50 m, fringing reefs: mean = 80.7 ± 10.83 m). A two-way comparison of mean distances moved between the factors management zones (closed and open to fishing) and time periods indicated that fish from closed zones moved further between recapture occasions than did fish from open zones ($p = 0.007$, Table 1; Fig. 2). Given that fish moved further on patch reefs than on fringing reefs, but no branded fish were resighted on patch reefs in the open zone, the analysis was repeated on a subset of data consisting only of fringing reef fish ($n = 56$, Table 1). The data subset (excluding patch reef data) resulted in reduced distances moved for fish from closed reefs (from 114.1 ± 12.57 to 99.6 ± 14.63 m, Fig. 2). Nevertheless, fish from fringing reefs closed to fishing did move further between recaptures than fish from fringing reefs open to fishing ($p = 0.044$, Table 1; Fig. 2).

Mean distances moved per day, as determined using ultrasonic telemetry, did not differ between habitats (*t*-test: $t_{[29]} = 0.91$, $p = 0.370$; patch reefs: mean = 200.6 ± 19.79 m, fringing reefs: mean = 178.0 ± 15.41 m) or between management zones (*t*-test: $t_{[29]} = 0.94$, $p = 0.356$; closed zone: 201.6 ± 17.49 m, open zone: 163.5 ± 13.19 m). However, the habitat and management zone patterns observed in the telemetry data appear similar to those in the mark–resighting data. A direct comparison of distances moved between the two methods indicated that the daily distances moved recorded through telemetry were larger than those obtained from the visual census surveys (*t*-test: $t_{[127]} = 5.46$, $p < 0.0001$). Fish carrying ultrasonic

Fig. 2. Effect of management zones on the distances moved by coral trout (time periods between resightings pooled). Comparison between management zones closed and open to fishing for complete data set (solid bars, $n = 93$) and fringing reef data subset (open bars, $n = 56$). Depicted are means ± SE. Means are indicated numerically.



transmitters moved, on average, 186.6 ± 11.96 m·day^{-1}, while freeze branded fish moved 94.9 ± 9.48 m between recapture occasions.

Reserve boundary movements

No cross-boundary movements between zones closed and open to fishing were recorded from freeze branded specimens. Of the 183 freeze branded fish released, only 10 individuals had their capture–release location within ~500 m either side of a reserve boundary (Fig. 1). Of these 10 branded specimens, four resightings were recorded during the two visual census surveys. The average distance moved between capture and resighting locations was 35.6 ± 1.64 m, with a range of 33.2–37.0 m.

During the 1993–1995 ultrasonic tracking study, eight coral trout equipped with transmitters had home ranges that straddled the reserve zone boundaries at Granite Head and at Chinamans Ridge (Fig. 3). On average, 31.5 and 68.8% of the home ranges of these fish were located in the open and closed zones, respectively (Table 2). Based on the number of times a fish was recorded on either side of the boundary, these fish spent, on average, 27.5 ± 10.81% of their time in the zone open to fishing (Table 2). The total number of boundary crossings recorded for the specimens being tracked ranged from 2 to 64 (mean = 27.5 ± 8.6) for tracking periods of 8 and 66 days, respectively (Table 2). The data were standardised to crossings per day and crossings per month (based on a 30-day month). The observed specimens averaged 0.5 crossing·day^{-1} or 15.3 crossings·month^{-1} (range from 3.6 to 29.1·month^{-1}, Table 2). Thus, coral trout tracked using ultrasonic telemetry would cross reserve boundaries bisecting their home ranges from once every 10 days to daily (Table 2).

Discussion

The present study attempted to quantify movement patterns of

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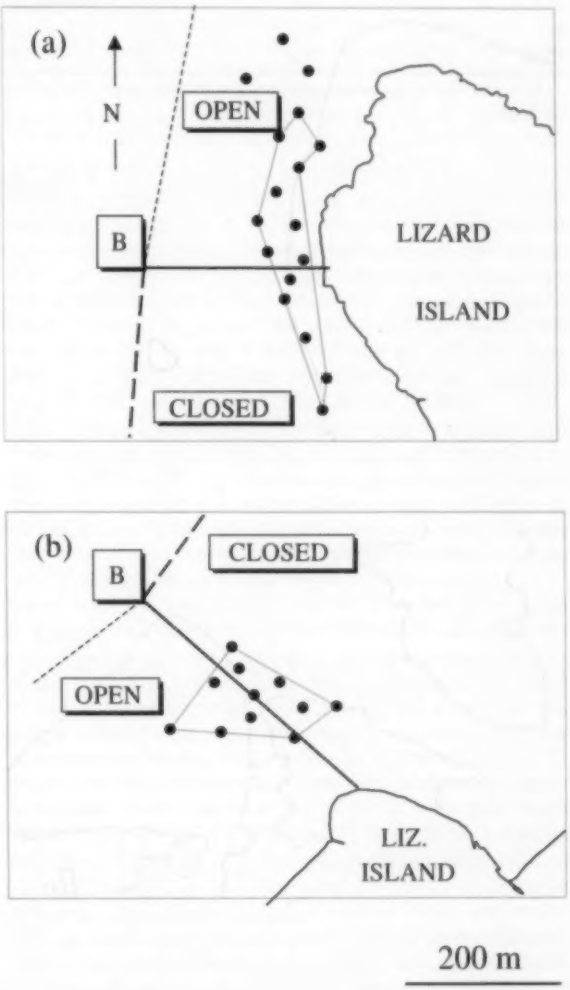
coral trout in relation to marine reserves, using UVC of individually marked animals and ultrasonic telemetry. The objectives were to assess the implications of adult movements for marine reserves and to compare movement information gained by the two methods.

This study presents the first data on bidirectional rates of movement across marine reserve boundaries of a coral reef fish of major fisheries significance. The data obtained for rates of boundary crossings by coral trout equipped with ultrasonic transmitters and with home ranges straddling marine reserve boundaries indicated that, on average, each coral trout crossed the boundary in either direction once every 2 days. Thus, these fish moved regularly across zone boundaries. Furthermore, the percentage of home range located in the open zones (mean = 31.2%) and the amount of time spent in the open zone (mean = 27.5%) corresponded relatively closely, suggesting approximately even use of unfished and potentially fished areas. The very low rates of exchange of fish across management boundaries, except for those fish with home ranges straddling the boundaries, argue that properly managed reserves may be very effective in protecting spawning stock biomass of coral trout populations on the Great Barrier Reef.

There are only limited empirical data on movements of postsettlement fish across management zone boundaries, potentially resulting in yield increases in areas outside the protected area (i.e., spillover). While some studies have attempted to use indirect measures of potential emigration and biomass export (e.g., Alcala and Russ 1990; Russ and Alcala 1996*a*), other studies have utilised mark–release–recapture techniques to evaluate movements of target species from protected to fished areas (e.g., Gitschlag 1986; Beinssen 1989) or modelled the potential effect of such movements on expected yields (e.g., Polacheck 1990; Russ et al. 1992; DeMartini 1993). However, only one study, to the authors' knowledge, measured emigration rates from a protected area directly. Attwood and Bennett (1994) tagged 11 022 fish over a 5.5-year period in a South African temperate water surf-zone marine reserve. Of the 9.1% total recaptures (research and fisheries), 17.8% had moved outside the 50-km-long protected area. However, all tagging effort was restricted to the inside of the reserve, with no tagging undertaken in adjacent waters.

Recent studies modelling the effect of marine reserves on the potential yield per recruit to areas adjacent to reserves suggested that moderate increases were possible outside of reserve areas (Polacheck 1990; Russ et al. 1992; DeMartini 1993). However, these studies indicated that yield per recruit would only increase if very high fishing mortalities existed outside the protected areas and rates of transfer of fish were high. Furthermore, DeMartini (1993) concluded that any enhancement in yield per recruit would be restricted to areas close to the reserve. This was supported empirically by Russ and Alcala (1996*a*), who showed that the increases in density and species richness of large predators associated with a protected area in the Philippines were most pronounced near (within a few hundred metres of) the reserve. In the present study, density was 20% higher in the closed areas, but this difference was not statistically significant, and the fishing pressure around Lizard Island is moderate (by Australian standards) to light (by developing country standards). Therefore, one would not expect net movements (displacements or relocations) of coral trout to areas outside the closed zones in response to density

Fig. 3. Representative examples of home ranges of two coral trout straddling marine reserve boundaries during 1993–1995. Circles represent position records of coral trout obtained using ultrasonic telemetry. Polygon outlines indicate minimum area polygon home ranges of specimens, short-dashed lines outline the zone open to fishing for commercial and recreational fishers, long-dashed lines outline the marine reserve (closed to fishing), and solid lines marked "B" indicate the locations of marine reserve boundaries. (*a*) Specimen PL 9 living at a location called Granite Head (see Fig. 1); (*b*) specimen PL 31 living at a location called Chinamans Ridge (see Fig. 1).



gradients. This ignores the potential for bait attraction during fishing, and as such represents a minimum rate of availability.

The lack of observed movements across reserve boundaries during the UVC surveys of freeze branded fish indicated strongly the necessity of concentrating considerable tagging effort near boundaries, followed by repeated, multiple recapture surveys. Due to the even distribution of initial capture as well as UVC effort in the present study, relatively little catch and recapture effort was allocated directly to boundaries.

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Table 2. Home range usage information determined with ultrasonic telemetry for coral trout whose home ranges straddled reef management zone boundaries at Lizard Island.

Fish	% home range		% time spent in open zone	No. of boundary crossings	Tracking duration (days)	Boundary crossings per day	Boundary crossings per month
	Open zone	Closed zone					
1	91.2	8.8	89.9	2	8	0.25	7.5
2	15.9	84.1	17.5	53	62	0.86	25.7
3	42.3	57.7	57.8	7	58	0.12	3.6
4	11.5	88.5	14.2	64	66	0.97	29.1
5	0.5	99.5	4.5	8	23	0.35	10.4
6	2.0	98.0	4.4	14	97	0.14	4.3
7	37.4	62.6	23.1	50	52	0.96	28.9
8	49.1	50.9	8.5	22	52	0.42	12.7
Mean	31.5	68.8	27.5	27.5	52.25	0.51	15.3

Note: Included are the percentage distribution of home ranges between zones, percentage time spent in the zone open to fishing, and rates of crossing of zone boundaries.

Beinssen (1989) obtained a similar result in a study of reef fish movement in relation to an existing reserve boundary at Heron Island (southern Great Barrier Reef). Of 273 coral trout tagged, 13.9% were subsequently resighted during underwater visual surveys 3–4 weeks after tagging. Of these resightings, 17% had moved less than 500 m, 8% had moved 500–1000 m, and 4% had moved 1000–1500 m from the tagging location. However, only one fish was recorded to have crossed the reserve boundary, moving at least 500 m from the closed to the open zone. In contrast, Davies (1995) reported that 35% of coral trout recaptures had moved out of the 1.5- to 2.5-km reef block in which they were released and attributed these movements to spawning behaviour (see also Zeller 1997*a*, 1998). Future investigations require concentration of capture and recapture effort close to boundaries, with gradual reduction in effort with distance from boundaries.

When attempting to interpret the observed difference in distance moved between the two different data collection methods (ultrasonic telemetry versus UVC of marked animals), it should be recognised that both represent different measures. Ultrasonic telemetry provided data on the average distance moved by each fish per day, based on several position records per day sampled over substantial time periods (mean 52.3 days, Table 2). Daily movements generally consisted of several movements back and forth throughout each specimen's home range (Zeller 1997*a*, 1997*b*). Thus, it is a measure of average total distance moved during a day. In contrast, the distance measure obtained through UVC of marked coral trout represented a once-off linear measure of displacement between two points in time at least 1 month apart, and thus should be lower than the average total distance moved per day. The findings supported this expectation. This result has important implications for design of studies to measure the spillover effect of marine reserves.

The comparison between UVC of marked animals and ultrasonic telemetry clearly illustrated that ultrasonic tracking provides more accurate information on patterns of movement by providing more data points per individual fish (Zeller 1997*a*, 1997*b*, 1998). However, given the cost and complexity of ultrasonic telemetry, a combination of detailed investigations of transmitter-equipped fish with a large marking study utilising repeated UVCs of marked animals to obtain densities and movement rates is recommended.

The densities of coral trout recorded in zones closed to fishing (5.5 ± 0.32 fish·1000 m^{-2}), while 20% higher than in open zones (4.6 ± 0.39 fish·1000 m^{-2}), were not significantly higher. Recent studies have provided evidence that marine reserves enhance the abundance, size, and hence biomass of numerous reef fish species (e.g., Russ and Alcala 1989, 1996*b*). In the present study, the overall density, as well as densities in the open and closed zones, compared well with other density estimates for coral trout from the Great Barrier Reef (review by Williams and Russ 1994). Williams and Russ (1994) indicated that, while the evidence was reasonably good that closing areas to fishing increased density, it was not unequivocal. Several studies detected no significant difference in density of coral trout between open and closed zones up to 8 years after closure (Williams and Russ 1994). It is possible that the general fishing pressure on reefs open to fishing on the Great Barrier Reef may not be high enough to significantly affect densities (Williams and Russ 1994). Furthermore, it has been suggested that infringements of zoning rules may be common enough in the line fisheries on the Great Barrier Reef to result in significant fishing pressure in supposedly closed areas (Williams and Russ 1994). Alternatively, the lower catches regularly reported by fishers in open zones may be the result of lower catchability, rather than lower densities in open zones (Beinssen 1989). This implies a behavioural difference of coral trout towards bait in open and closed areas. Fishing pressures around Lizard Island could be considered moderate, consisting predominantly of recreational fishing effort (D.C. Zeller, personal observation). Infringements of existing zoning rules were not observed to occur regularly during this study, possibly due to the close proximity and high activity levels of the local research station and resort. Nevertheless, the experimental line capture data obtained in this study strongly support the notion of reduced levels of catchability of coral trout in the zones open to fishing, with CPUE 2.58 times lower in open zones than in closed zones, despite densities not being significantly different. The CPUE data recorded in the present study were slightly lower than those recorded by Davies (1995). Davies (1995), using commercial fishers for a large-scale tagging study in the central section of the Great Barrier Reef, reported CPUE of 2.26–7.44 coral trout·$line^{-1}$·h^{-1}. Thus, the results obtained in the present study clearly support the previously reported observations and may explain the common

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concern of reduced catches on heavily fished reefs expressed by line fishers.

Coral trout in closed zones moved greater distances between capture and resighting than those in zones open to fishing. The observed differences cannot be explained simply by population structure differences, as densities, although slightly lower in open zones, did not differ significantly between zones. Also, the size distribution of coral trout in the general study area did not differ significantly between management zones (Zeller 1997*a*). Furthermore, as ultrasonic tracking indicated, home ranges did not vary significantly in area, length, or width between management zones (Zeller 1997*a*). Given the very small sample size of visual resightings in open zones ($n = 19$) and the fact that the UVC results for distances moved were based on two points in time (capture and resighting), one would have to treat this result with caution. Fish tracked with ultrasonic telemetry did not differ significantly in the distance moved per day in either zone. However, ultrasonic telemetry suggested that fish moved nearly 20% greater distances in closed areas. It is difficult to hypothesise an ecological or biological reason for these results, particularly given that the population structure and density appeared similar between zones.

The lack of a clear relationship between distances moved and size of fish recorded during the UVC surveys indicated that size alone was not a good indicator of movement range for coral trout. Patch reef fish moving further than fringing reef fish is possibly due to differences in the shape of home ranges of coral trout. Home ranges of fish living on patch reefs are wider but not longer than home ranges on fringing reefs (Zeller 1997*b*). Thus, it is possible to resight fish on patch reefs at greater distances from the initial capture location than is possible for fringing reef fish. Ultrasonic telemetry data support this, with mean distances moved per day not differing between the two habitats. Thus, on average, fish in either habitat moved similar distances per day, but there was more chance of resighting freeze branded fish on patch reefs further from the location of capture than was the case for fish on fringing reefs.

Conclusions and recommendations

This study recorded very low rates of flux of postsettlement coral trout across marine reserve boundaries at Lizard Island. However, ultrasonic telemetry did indicate that individuals with home ranges incorporating boundaries moved regularly between zones. While there were no significant differences in densities of coral trout between open and closed zones, the CPUE was considerably higher in closed zones than in open zones. Thus, clear differences existed in catchability of coral trout between management zones, providing further evidence that reported concerns regarding reduced catches by fishers on the Great Barrier Reef may partly reflect behavioural changes in targeted species. It is strongly recommended that estimation of flux rates with respect to marine reserves be based on controlled manipulations of fishing effort and incorporate a dual approach, combining ultrasonic telemetry to provide detailed information on movements with large-scale marking and visual resighting or recapture studies, with strong emphasis on intensive, multiple resighting opportunities near boundaries.

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Use of lipofuscin for resolving cohorts of western rock lobster (*Panulirus cygnus*)

Matt Sheehy, Nicolavito Caputi, Christopher Chubb, and Mark Belchier

Abstract: We evaluated lipofuscin age pigment as an approach to age determination of western rock lobster (*Panulirus cygnus*). Lipofuscin was measured using fluorescence image analysis of olfactory lobe sections from 184 juvenile lobsters from coastal nursery reefs at Seven Mile Beach, Western Australia. Modal analysis of a lipofuscin concentration–frequency histogram for this sample suggested the presence of additional age-classes, not apparent in conventional size–frequency distributions but expected to be present from earlier tag–recapture studies. Modal progression suggested a remarkably constant average lipofuscin accumulation rate of 0.31% by volume per year in the wild lobsters, which was supported by blind-trial measurements of lipofuscin in known-age laboratory-reared specimens. Lipofuscin-based age estimates, indicating that most juvenile lobsters are 3–5 years old immediately prior to recruitment to the fishery at 76 mm carapace length, agree with the long-established relationship between puerulus settlement and lobster catch 3–4 years later. Results indicate that the lipofuscin ageing technique will permit independent assessment of current population parameter estimates and be useful for determining sexual and regional differences in these for the western rock lobster.

Résumé : Nous avons évalué la possiblité d'utiliser la lipofuscine, pigment indicateur d'âge, comme méthode de détermination de l'âge de langoustes diablotins (*Panulirus cygnus*). Nous avons mesuré la lipofuscine grâce à une analyse d'images par fluorescence de sections du lobe olfactif de 184 langoustes juvéniles provenant de nourriceries de récifs côtiers situés à Seven Mile Beach, dans l'ouest de l'Australie. D'après l'analyse modale d'un histogramme des fréquences de la concentration de lipofuscine dans cet échantillon, il y aurait des classes d'âge supplémentaires, non apparentes dans les distributions classiques des fréquences de taille, mais suggérées par des études antérieures de marquage–recapture. La progression modale fait ressortir un taux d'accumulation de lifpfuscine remarquablement constant de 0,31% en volume par année chez les langoustes sauvages, résultat qui a éte appuyé par des mesures à l'aveugle de la lipofuscine chez des sujets d'âge connu élevés en laboratoire. Les estimations de l'êge fondées sur la lipofuscine, qui indiquent que la plupart des langoustes juvéniles ont entre 3 et 5 ans juste avant leur recrutement à la pêche (longueur de carapace 76 mm), corroborent la relation déterminée depuis longtemps entre l'établissement des puerulus et les captures de langoustes 3 ou 4 ans plus tard. Les résultats indiquent que la technique d'âgeage à la lipofuscine permettra d'évaluer de façon indépendante les estimations actuelles des paramètres de la population, et facilitera la détermination des différences sexuelles et régionales dans ces paramètres chez les langoustes diablotins.
[Traduit par la Rédaction]

Introduction

In the 1995–1996 season, 9.8 million kg of western rock lobster (*Panulirus cygnus*) was landed, worth Aus$223 million. This lobster continues to support Australia's most valuable single-species fishery. The resource is heavily harvested, and the primary policy objective of the managing authority is to ensure that fishing effort is contained at sustainable levels. This produces a need for continuous and intensive research involving revision of the fishery data, refinement of catch forecasting, and population modelling (Australian Government 1991).

Data on population age composition are desirable for the provision and interpretation of information on growth, recruitment, migration, maturation, longevity, and mortality in the lobster. Morgan (1980) noted the difficulties in population dynamics modelling for palinurid lobsters when using approaches analogous to those for finfish, due to the lack of suitable age estimation methods. Crossland et al. (1988) emphasized that establishment of a precise age criterion had wide and important implications for research and management of the western rock lobster fishery and, by extension, for lobster fisheries elsewhere. Conventionally, modal analysis of length–frequency histograms has been used to age the lobsters. Two-and 3-year-old cohorts can usually be detected by eye in these histograms (Chittleborough 1970, 1976), and computer-based maximum-likelihood procedures may resolve additional modes attributed to 4-and 5-year-old lobsters (Phillips 1990). However, older cohorts are not detectable. When placed in perspective against the whole life span, which in this species may exceed 27 years, the applicability of the method is quite limited.

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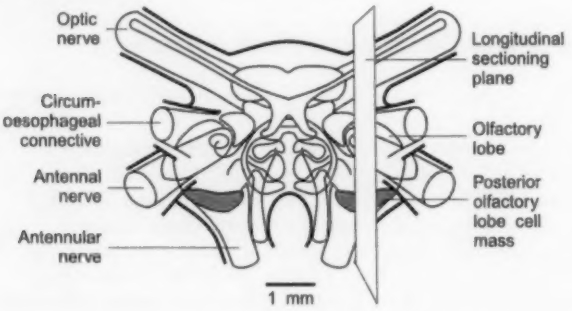
Fig. 1. Schematic view of the brain of a palinurid lobster (modified from Sandeman et al. 1993) showing the location of the posterior OLCM and plane of sectioning used for lipofuscin measurement in the present study.



The possibility of directly ageing palinurid lobsters is now not as remote as it once seemed. A recent molecular approach has consistently found that the neuronal accumulation of insoluble autofluorescent lysosomotropic compounds, collectively termed lipofuscin, is a better predictor of chronological age than body size in various decapods (e.g., Sheehy 1990*a*; Sheehy et al. 1994, 1996; Belchier et al. 1998). Where a significant portion of the life span has been examined, chronological age explains 92–93% of the variance in lipofuscin concentration. Lipofuscin concentration is typically less variable within age groups than carapace length or body mass and its accumulation rate declines less markedly with advancing age than growth rate.

These results have indirectly supported Ettershank's (1983) original concept of elucidating population age structure for a crustacean, the Antarctic krill (*Euphausia superba*), by lipofuscin concentration–frequency analysis. Distinct annual spawning pulses followed by steady accumulation of lipofuscin in each ageing cohort are assumed to be responsible for regular clustering patterns in lipofuscin concentration in a process analogous to that responsible for modes in size–frequency histograms. Following revision of the original lipofuscin measurement procedure (Sheehy 1989, 1990*b*), Sheehy et al. (1994) used data from captively-reared known-age freshwater crayfish (*Cherax quadricarinatus*) to simulate size–frequency and lipofuscin concentration–frequency distributions for this species. These simulations clearly suggested that better cohort separation would be obtained using lipofuscin and led to the hypothesis that lipofuscin offered a new basic dimension for detection of year-classes in wild crustacean populations. The present study is a field test of this hypothesis for the western rock lobster. We also explore the potential usefulness of the technique for deriving the lobster's population parameters.

Materials and methods

Conventions for specifying age

Two formats exist for specifying age in the literature: either as discreet, more or less approximate values, i.e., 1 year, 2 years, 3 years, etc., or, particularly when referring to cohorts in frequency histograms, in the format "years+", e.g., 0+, 1+, 2+, etc. This latter format implies a range of possible ages in each group. For example, the 0+ mode consists of individuals 0–1 year old, 1+ contains individuals 1–2 years old, and so forth. This is necessary because, not only is there a natural spread of ages in each mode, but the average age of individuals in each mode differs depending on what time of year the sample is taken. It will increase along with the time elapsed since the last annual breeding or recruitment pulse. For the western rock lobster, the "birth date" of all individuals is customarily taken as January 1, which corresponds approximately to the middle of the period when the majority of eggs hatch (Morgan et al. 1982). To assist comparisons with the literature, both age formats are used in this paper. For our samples, which were taken late in the year (October and December), individuals classified into the 1+ cohort will have an average age of almost 2 years, individuals in the 2+ cohort will have an average age of almost 3 years, and so on.

Lobster samples

Three groups of western rock lobster were used for this study. The primary group of 184 juvenile lobsters of unknown age was collected in mid-October 1993 from the gazetted research area on the inshore coastal reefs at Seven Mile Beach (SMB) (29°08′S, 114°54′E), Western Australia. Average annual sea temperature here is ~21.5°C. A depth-stratified sampling procedure with uneven effort at three different locations over 2 days provided lobsters from a range of sizes and ages. Lobsters were collected 100 m offshore in water ~2 m deep, 200 m offshore in water ~4 m deep, and 1 km offshore in water ~20 m deep. Collapsible "parlour and bedroom" pots with small mesh were used to trap lobsters at the two shallower locations and standard cane pots were used at the deeper site. The sample used for analysis contained 98 females and 86 males and its carapace length (CL) composition was as follows: 30.0–39.9 mm, $n = 7$; 40–49.9 mm, $n = 38$; 50–59.9 mm, $n = 47$; 60–69.9 mm, $n = 44$; 70–79.9 mm, $n = 48$.

The second group of 20 lobsters of unknown age was collected in February 1993. The CL range of these lobsters was 58.5–153.4 mm and they included both immature and mature males and females. The smaller lobsters were obtained from coastal reefs near the Western Australian Marine Research Laboratories (WAMRL), North Beach, Perth, Western Australia. The larger lobsters were obtained from a local processing plant, Western Australian Seafood Exporters (WASE), Osborne Park, Perth. The precise geographical origin of the larger lobsters was not known.

The third group of 18 lobsters of known age was sampled in December 1995. This sample included six recently metamorphosed postpueruli (9 mm CL) considered to be about 1 year of age, although exact ages may vary by a few months (Morgan et al. 1982). There were also 12 juveniles (six females and six males) that had been reared for up to 6 years from the time of settlement in tanks at WAMRL. Water supply to these tanks was pumped from the ocean through an open circulation system. Its temperature closely approximated local sea temperatures, averaging ~20.6°C annually, and was similar to that at SMB. These lobsters were fed daily on a diet of mussels, abalone, or fish. At sampling, they were aged as follows: ~2 years (47.0–52.8 mm CL), $n = 4$; ~3 years (66.5–71.8 mm CL), $n = 4$; ~7 years (85.3–92.6 mm CL), $n = 4$. Investigators undertaking lipofuscin measurements on these individuals did not have prior knowledge of their precise age or length composition.

Lipofuscin identification and measurement

Lobsters were immobilized in ice water prior to decapitation. After removal of antennae, eyes, and cephalic horns, whole heads were thoroughly rinsed in seawater and fixed in 500-mL volumes of 4% formaldehyde in seawater chilled to 4°C. Following fixation for at least 48 h, brains were dissected out, longitudinally halved with a sharp blade to allow solvent penetration, and the right halves processed for wax embedding using standard histological procedures (Sheehy 1989; Sheehy and Wickins 1994). Embedded material was sectioned so as to obtain serial longitudinal slices, 6 µm thick, through the posterior olfactory lobe cell mass (OLCM) (Fig. 1). For identification of lipofuscin, slide-mounted sections were examined for characteristic granular yellow autofluorescence under 450 nm excitation

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and identically distributed Sudan Black positive staining according to methods described previously (Sheehy 1989; Sheehy and Wickins 1994). For lobsters of unknown age, lipofuscin in sections of the OLCM was quantified by image analysis of fluorescence micrographs generally as described in Sheehy (1990*b*). Due to the more homogeneous distribution of lipofuscin in the OLCM of western rock lobster, the previous procedure was adapted so that 10 roughly equidistant sections were selected for measurement from the entire cell mass rather than only the region containing the olfactory globular tract as in previous studies. Alternative confocal microscope and image analysis facilities were used for measurement of lipofuscin in the OLCM of known-age lobsters. These were generally as described in Sheehy et al. (1996). Approximately 27 sections were sampled equidistantly through the OLCM. Excitation at 488 nm was used and the image scan window was zoomed by a factor of 1.05 to give an effective magnification and image field width (205 μm) identical to that used on previous equipment. To adjust for biases due to (*i*) images occasionally containing unusually large granular aggregations of lipofuscin and (*ii*) variation in the total area of suitable tissue present in each image, lipofuscin concentrations for individual lobsters were calculated as weighted geometric averages of the percentage area fractions of lipofuscin in each of the replicate OLCM images. Weights consisted of the total cross-sectional area of suitable tissue (globuli neurone cell bodies) in each image. Each weighted geometric mean, $\overline{Y}_A$, was calculated according to the following formula

$$\overline{Y}_A = 10^{\left(\frac{\sum\left[\log\left(\frac{L\times 100}{A}+c\right)\times A\right]}{\sum A}\right)} - c$$

where L is the total cross-sectional area of lipofuscin in an image (square micrometres), A is the total cross-sectional area of suitable tissue in an image (square micrometres), and c (a constant) = 0.04. This weighted geometric average is subsequently referred to as the lobster's lipofuscin concentration and, following stereological convention, is considered equivalent to the volume fraction in percentage (%VF) of lipofuscin in the OLCM.

Frequency histograms, modal analyses, and age-class designations

A standard length–frequency histogram was constructed from the CL data for the SMB lobsters. A class interval of 3 mm was selected for the present analyses, following Chittleborough's (1976) practice of applying a sliding average across 3 × 1 mm intervals to smooth distributions. A lipofuscin concentration–frequency histogram was also constructed. Appropriate class interval width depends on sample size and may have a marked effect on histogram appearance, so for valid comparison, class intervals in the latter were chosen so that the bulk of the data lay within the same number of class intervals as in the length–frequency histogram.

To define potential cohorts in the histograms, normal components were fitted to modes using ELEFAN (ICLARM, Makati, Philippines). Only modes that were visually obvious and well separated were fitted. Modal separation indices were, in all cases, well above the critical value of 2. Goodness of fit of the predicted and observed frequency distributions was confirmed using χ^2 tests. In each histogram, where adjacent modes overlapped, the expected contribution from each mode to each class interval was calculated. As no class interval was expected to be composed of precisely equal contributions from two or more modes, it was possible to designate individuals within each class interval to a particular mode on the basis of highest likelihood.

For the length–frequency histogram, modes were assigned to particular age-classes of lobsters based on conventions in the previous literature (Chittleborough 1970, 1976; Ford et al. 1988; Edgar 1990; Jernakoff et al. 1994). For samples undertaken around October at SMB, lobsters with CLs of ~25–38 mm are typically considered to be 1+ individuals, 38.1–55.0 mm to be 2+, 55.1–68.0 mm to be 3+, and >68.1 mm to be 4+. For the SMB lipofuscin concentration–frequency histogram, the assumption that the regular modes present reflected yearly cohorts, with a temporal progression indicating the average annual lipofuscin accumulation rate, was verified by comparison with data from the known-age WAMRL lobsters reared under comparable thermal conditions. This reference sample of known-age lobsters was also used to assign the chronological age of individuals in the first mode in the SMB lipofuscin concentration–frequency histogram. The linear regression fitting the modal progression in this histogram was then used to back-calculate specific ages for all lobsters in the SMB and WASE samples.

Lipofuscin-based population parameter estimates

To estimate growth parameters, the relationships between lipofuscin-estimated age and CL in male and female lobsters were fitted with von Bertalanffy models:

$$CL_t = CL_{max}[1 - e^{-k(t-t_0)}]$$

where CL_t is carapace length (millimetres) at age t (years), CL_{max} is the mean carapace length of the oldest lobsters, k is a curvature parameter defining how rapidly CL_{max} is reached, and t_0 is the theoretical age at which CL = 0. Parameter values were optimized by standard iterative least-squares estimation procedures. All models were constrained so as to pass through a plausible length-at-metamorphosis-age point (9 mm CL at 1 year). CL_{max} values for males and females were approximated by fitting curves through the length-at-estimated-age data from the large WASE lobsters. These estimates were then used as constrained parameter values in equations fitting the relationship between length and estimated age in the male and female SMB lobsters for which no very large individuals were present. The latter equations were therefore fitted for k only.

To calculate a loss rate analogous to the instantaneous rate of total mortality, the relative strengths of the successive modes in the lipofuscin concentration–frequency histogram were examined. Because of the uneven sampling effort used, the length distribution of the sample was biased, with disproportionately low frequencies of smaller individuals. Hence, the age structure of the sample was also biased. To correct for this artefact, the length–frequency distribution was first adjusted according to the density data presented by Phillips (1990, his table 2) to the average relative proportions of lobsters in the 2+, 3+, and 4+ modal groups found from surveys for the years 1967–1981. These relative proportions were 1 for 2+, 0.68 for 3+, and 0.15 for 4+. The ratio of these values over the observed relative modal strengths in the SMB length–frequency histogram were 1, 0.39, and 0.12, respectively, and these values were used to adjust the frequency of individuals in the SMB length modes. Since the lipofuscin-estimated age distribution of individuals in each length-class was also known, the weighting procedure also adjusted the number of individuals in each lipofuscin-estimated age-class to the proportions expected in a sample with a more representative length composition.

The declining relative abundances of SMB lobsters with increasing age was modelled using the equation

$$R_t = R_{t_s}\, e^{-C(t-t_s)}$$

where R_t is the estimated number of individuals at time t (years), relative to the number of ~3-year-olds (=2+ years, the peak age-class due to sampling bias against smaller individuals), and R_{t_s} is the number of individuals at the time of settlement, t_s (=0.8 year), again relative to the number of 3-year-olds. Parameter C indicates rate of loss analogous to the instantaneous rate of total mortality, Z. In the present study, on migratory juveniles, this parameter defines loss due to both natural mortality and emigration.

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Results

Length–frequency analysis

There was no significant difference between the observed CL–frequency distribution for SMB lobsters and its fitted normal components (χ^2 test, $p > 0.05$) (Fig. 2*a*). In accordance with the usual practice, modes at CLs of 30.5, 44.9, 58.1, and 72.6 mm were designated as 1+, 2+, 3+, and 4+ age-classes. Modes appeared well resolved with relatively high separation indices (Table 1). No age-classes older than 4+ were apparent. The distribution was artificially truncated at the upper end due to sampling for individuals of <80 mm CL only.

Lipofuscin concentration–frequency analysis

Characteristic lipofuscin granules were found in the posterior olfactory lobe and other cell masses in the brains of all lobsters examined, except for postpueruli. There was no significant difference between the observed lipofuscin concentration–frequency histogram for SMB lobsters and its fitted normal components (χ^2 test, $p > 0.05$) (Fig. 2*b*). Fitted modes were again well resolved with relatively high separation indices (Table 1). There was close correspondence in the modal positions for males and females, although the eight individuals with highest lipofuscin concentrations were all females.

There was overlap of OLCM lipofuscin concentrations in WAMRL lobsters of different known ages, but the relationship between lipofuscin concentration and age was highly significant ($p < 0.0001$) (Fig. 3). The linear regression defining this relationship had a slope of 0.209 ± 0.112%VF·year^{-1} (mean ± 95% confidence limits) and an R^2 of 80%. ANOVA indicated that there was no significant effect from sex on lipofuscin concentration ($p = 0.77$) after allowing for the effects of differences in age.

The minimum average difference between the mean lipofuscin concentrations in each mode of the SMB lipofuscin–concentration histogram (Fig. 2*a*) and the mean lipofuscin concentrations in each known age group of WAMRL lobsters (Fig. 3) was achieved by assigning the first mode to the 1+ cohort. As well as 2–4+ modal groups, additional 5+ and 6+ cohorts were resolved in the lipofuscin concentration–frequency histogram, with indications of a very small number of 7+ individuals. The observed SMB modal progression indicated an almost constant rate of lipofuscin accumulation (0.309%VF·year^{-1}, $R^2 = 99.7\%$) commencing around the time of settlement (Fig. 3, dotted line). The equation defining the relationship is

$$L = 0.309t - 0.351$$

where L is OLCM lipofuscin concentration (%VF) and t is age (years).

Lipofuscin-based population parameter estimates

Length-at-estimated-age data for SMB lobsters showed considerable scatter (Fig. 4). The best-fitting von Bertalanffy growth curve segment for male SMB lobsters (Fig. 4*a*; Table 2) intercepted the minimum legal size, 76 mm CL, at 4.8 years. Growth of female SMB lobsters was slower than males, with the growth model intercepting minimum legal size 1 year later at 5.8 years (Fig. 4*b*). Variation in length-at-age and age-at-recruitment was estimated to be higher for female lobsters than for males. For SMB juveniles as a whole, average age at entry to the fishery in 1993 was estimated to be 5.3 years, or about 4.3 years postsettlement. Note, however, that the regression lines represent the best fit to the average trends in the data. The distribution of data points for males, and more so for females, is right-skewed so that the modal age at reaching legal size is slightly less than indicated by the regression intercepts. A lipofuscin-estimated age–frequency distribution of sampled SMB lobsters of 68–80 mm CL at, or approaching (within one or two moults, or ~1 year), minimum legal size demonstrates this point (Fig. 5). The modal age of the lobsters in the histogram is around 4 years whereas the average age is 5 years. Males recruit about a year earlier than females, with a significant spread of slower-growing older pre-recruits present, particularly females.

The sample size of WASE lobsters was limited, but these data again suggested that male lobsters had a slightly faster growth rate than females (Fig. 4; Table 2). Average growth rate for WASE lobsters was estimated to be slightly higher than that of SMB lobsters. The best-fitting growth curve for male WASE lobsters intercepted minimum legal size at an age of 4.1 years (Fig. 4*a*) and for females at 4.8 years (Fig. 4*b*). Estimated growth curves for male and female SMB and WASE lobsters are placed in context with previously published, conventionally derived growth curves for the western rock lobster in Fig. 6 (see Discussion).

Individuals classified as 1+, 2+, and 3+, based on their lipofuscin concentrations, were located in all four length modes (Fig. 7*a*), and those classified as 4+ and 5+ were located in the larger three length modes. Individuals classified as 6+ and 7+, based on their lipofuscin concentrations, were located in the larger two length modes. Individuals with higher lipofuscin concentrations tended to be larger. The data therefore indicated a broad consensus between length-estimated and lipofuscin-estimated ages. However, each length group contained individuals of a wide range of lipofuscin concentrations, suggesting considerable variance in the relationships between size, lipofuscin concentration, and age. These trends are also apparent in Fig. 4. After weighting the modes to simulate a more typical length composition for SMB (Fig. 7*b*), the contribution of larger individuals to the lipofuscin-estimated age composition of the sample was considerably reduced. The effect can be seen more clearly by comparison of Figs. 8*a* and 8*b*; most obviously the height of the 3+ mode was reduced relative to the 2+ mode. The smallest lobsters were located in the lowest three lipofuscin concentration modes (Fig. 8*a*). All lipofuscin modes except for the 6+ and 7+ regions contained representatives from all but the smallest length group. Lipofuscin-estimated 6+ and 7+ individuals were derived from 3+ and 4+ length modes. Again, a high degree of variation in the relationships between size, lipofuscin concentration, and age was indicated. Despite this, however, lipofuscin-estimated ages 1+ to 4+ differed from length-estimated ages by no more than one age-class in 85% of cases.

In the SMB sample, lipofuscin-estimated 3-year-old males were more abundant than 3-year-old females, but thereafter, females predominated in each age group (Fig. 9). The widest difference in sex ratio occurred in estimated 5-year-olds, when females were more than twice as abundant as males. In older age groups, numbers of males and females became more similar, with only a slight predominance of females. At 6 years of age, the number of lobsters present was only about 13% of that

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Fig. 2. (*a*) Length–frequency distribution for 184 juvenile western rock lobster caught at SMB in mid-October 1993 using a stratified sampling procedure. Also shown are sex composition of modes, fitted normal components, and conventional cohort designations 1+ to 4+. The vertical dashed line represents minimum legal size (76 mm CL). (*b*) OLCM lipofuscin concentration–frequency distribution for the same SMB sample. Also shown are sex composition of modes, fitted normal components, and cohort designations 1+ to 6+ based on a calibration using known-age lobsters (Fig. 3). The arrow shows the position of possible 7+ individuals.

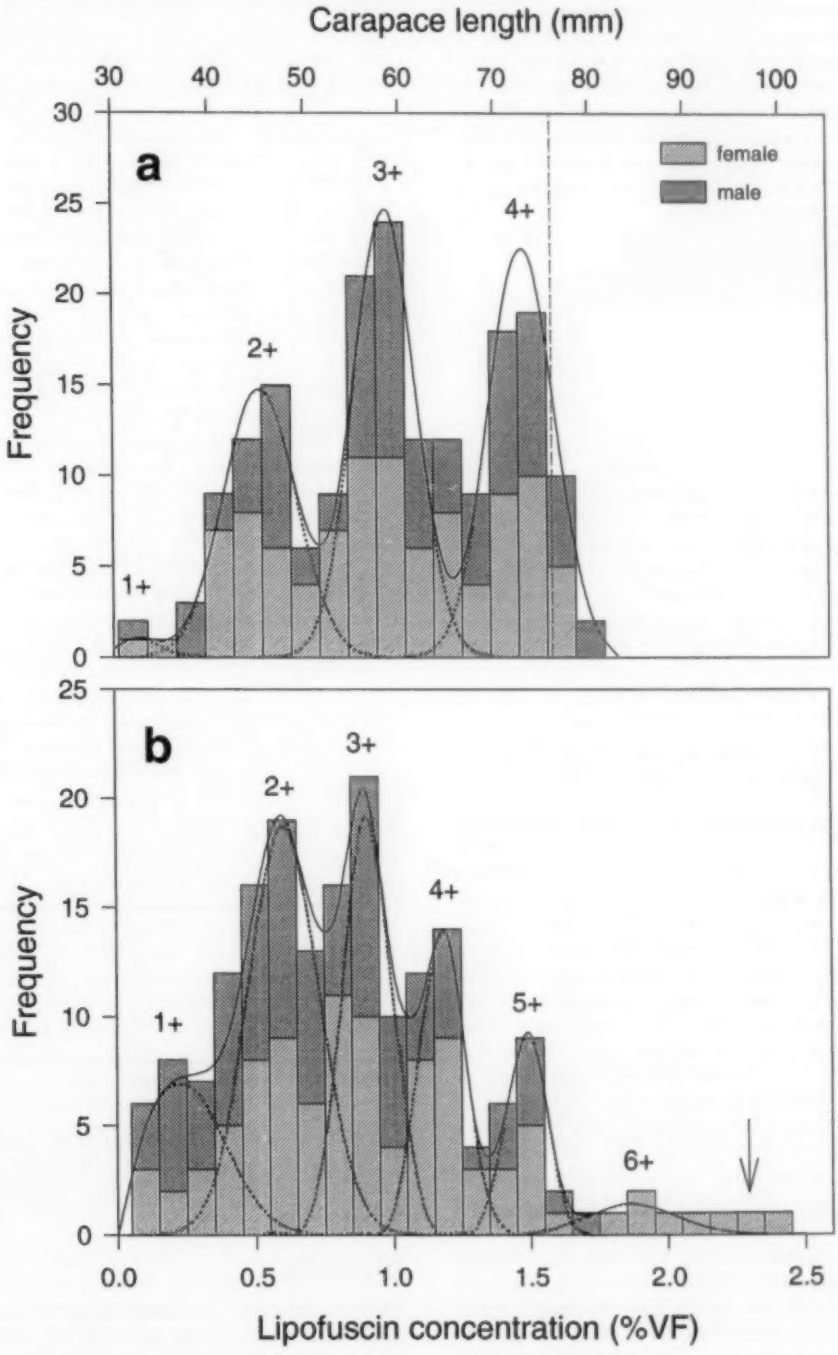

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Fig. 3. Comparison of lipofuscin accumulation in known-age WAMRL lobsters with that predicted for SMB lobsters. For WAMRL lobsters (solid circles), data are fitted with a linear regression (solid line) with 95% confidence (dashed lines) and prediction (dashed–dotted lines) intervals. Also shown are WAMRL age group means (open circles) and postpueruli (triangles). For SMB lobsters, the progression of lipofuscin concentrations modes (squares) through putative age-classes (Fig. 2*b*; Table 1) is fitted with a linear regression (dotted line). Error bars represent estimated 95% confidence intervals for age group means. Broken axes ranges place available data in context against the maximum known longevity of western rock lobster (27 years) and the highest average neuronal lipofuscin concentration so far encountered for a crustacean (~7%VF).

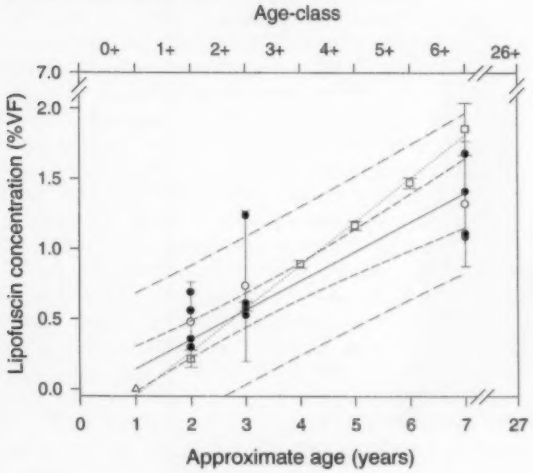

Table 1. Results summary for modal analyses of length–frequency and lipofuscin concentration–frequency histograms for SMB lobsters.

Variable	Group designation	$\bar{Y}$	s	n	Apparent separation index
CL (mm)	1+	30.50	3.6	3.3	—
	2+	44.90	3.8	46.7	3.9
	3+	58.10	3.2	66.7	3.8
	4+	72.6	3.4	65.3	4.4
Lipofuscin concentration (%VF)	1+	0.22	0.15	26.9	—
	2+	0.59	0.13	60.0	2.7
	3+	0.90	0.09	42.4	2.8
	4+	1.17	0.08	28.9	3.2
	5+	1.47	0.07	16.8	4.0
	6+	1.86	0.16	5.6	3.3

at 3 years of age. No males estimated to be older than 7 years were present and only a very few females persisted beyond this age. The best-fitting trend lines for the data as a whole slightly overestimate relative abundances for these older age groups. Estimated loss coefficients, C, were 0.86 for SMB males, 0.58 for SMB females, 0.70 for all SMB, 0.95 for Phillips (1990) SMB ("estimate 1" density data), and 0.65 for Phillips (1990) SMB ("estimate 2" density data). Based on the present sample and lipofuscin age estimates, R_{t_0} was calculated as 4.5, i.e., the density of settled puerulus is about 4.5 times the density of 3-year-olds on the reef.

Fig. 4. Lipofuscin-estimated age versus CL with preliminary von Bertalanffy curves for (*a*) male and (*b*) female SMB (solid circles, solid line) and WASE (open circles, dashed–dotted line) lobsters. The horizontal dashed line represents minimum legal size.

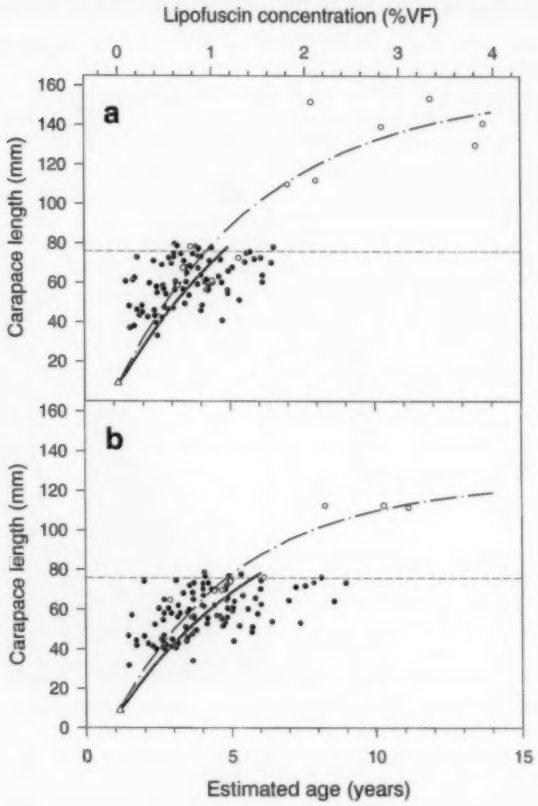

Table 2. Parameter values for preliminary von Bertalanffy models defining the relationships between CL- and lipofuscin-estimated age for male and female SMB and WASE lobsters.

Origin	Sex	CL_{max} (mm)	k (year^{-1})	t_0 (years)
WASE	Male	158.80	0.20	0.85[a]
	Female	125.00	0.23	0.78[a]
SMB	Male	158.8[a]	0.17	0.85[a]
	Female	125[a]	0.19	0.78[a]

[a]Values constrained during model fitting.

Discussion

Modal separation in our SMB length–frequency data is much better than that generally portrayed in the literature (Chittleborough 1970, 1976; Ford et al. 1988; Jernakoff et al. 1994). This may be due to the nonrandom sampling protocol used to optimize representation from a wide range of age groups (see

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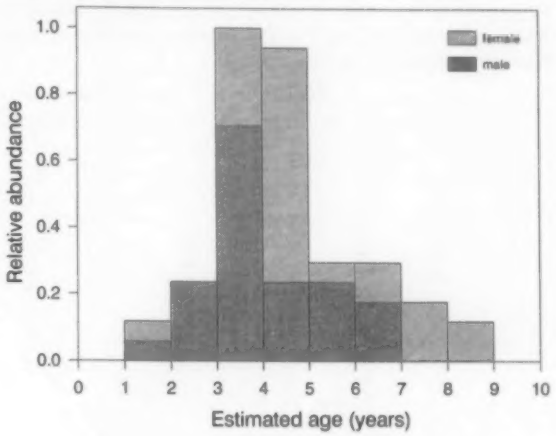
subsequent discussion). However, the number and position of modes in this histogram are comparable with those reported in the literature. It therefore forms an adequate example for comparison with the novel lipofuscin concentration–frequency histogram. Size ranges of the known-age laboratory-reared WAMRL lobsters bore little relationship to those expected from the present size–frequency histogram (Fig. 2*a*) or published growth data for SMB (Fig. 6), highlighting the pitfalls in extrapolation of laboratory-derived information on growth to the natural environment.

The lipofuscin concentration–frequency histogram for SMB juveniles showed a total of six resolvable modes. The regular shape and very even spacing of these modes are evidence that they are not simply random artefacts. The constant rate of modal progression suggests a linear average lipofuscin accumulation rate in the OLCMs of wild juvenile lobsters. For proper interpretation of this histogram, particularly the identity of the first mode, evaluation against data from the known-age WAMRL lobsters proved critical. Sheehy et al. (1994) showed that, for younger age groups of freshwater crayfish (*C. quadricarinatus*), lipofuscin accumulation rate under field conditions was almost identical to that in individuals kept in the laboratory at comparable temperatures. Relevant and significant effects on lipofuscin accumulation rate from sex, brood origin, diet, oxygenation level (Sheehy 1990*b*), and growth (Sheehy et al. 1994, 1995*b*, 1996; de Kerros et al. 1995; Wahle et al. 1996) have not been demonstrated. The best consensus between the laboratory and SMB data suggested that the first mode in the latter consisted mainly of 1+ individuals (Fig. 3). However, this mode was primarily composed of individuals of a length typically considered, at SMB, to represent 2+ individuals and older (Fig. 8*a*). A plausible explanation for this apparent discrepancy is that the composition of the lowest lipofuscin concentration mode is disproportionately biased toward relatively large fast-growing late 1+ juveniles. Growth rates this high occurred in the present laboratory-reared juveniles (Fig. 6) and have been reported in the literature (Chittleborough 1976; Phillips et al. 1977). The smaller individuals in this 1+ age-class are not trappable (Chittleborough 1974; Chittleborough and Phillips 1975; Phillips 1990). The corollary of this is that the mode designated as 2+ in SMB length–frequency histograms is not temporally homogeneous but includes many large late 1+ individuals also (Fig. 7*a*).

Despite a small sample size and age range, the present laboratory sample indicated that the approximate rate of accumulation of lipofuscin in the OLCM of western rock lobster reared under thermal conditions similar to those at SMB was 0.209%VF·year^{-1}. From modal progression, the mean lipofuscin accumulation rate for SMB lobsters was 0.309%VF·year^{-1}. This rate is closer to that recorded for long-lived European lobster (*Homarus gammarus*) (~0.07%VF·year^{-1}) (Sheehy et al. 1996) and freshwater crayfish (*Pacifastacus leniusculus*) (~0.2%VF·year^{-1}) (Belchier et al. 1998) than to that for relatively short-lived freshwater crayfish (*C. quadricarinatus*) (~2.0%VF·year^{-1}) (Sheehy et al. 1994). This is as expected from previous observations that the rate physiological ageing, as manifested by lipofuscin accumulation, may be inversely proportional to longevity (Sheehy et al. 1995*b*). When the accumulation rates for WAMRL (0.209%VF·year^{-1}) and SMB (0.309%VF·year^{-1}) lobsters are placed in perspective against the very wide range of lipofuscin accumulation rates that has

Fig. 5. Lipofuscin-estimated age–frequency distribution for SMB lobsters of 68–80 mm CL at, or approaching (within one or two moults, or ~1 year), minimum legal size.



so far been encountered in the crustacean OLCM (0.0005–4%VF·year^{-1}) (Sheehy et al. 1994; M. Sheehy, unpublished data), it is extremely unlikely that their similarity is coincidental. The true range of lipofuscin concentrations in each age group of SMB lobsters cannot be determined from the present data, but a comparison of Figs. 2*b* and 3 indicates that the spread of lipofuscin values within each mode is similar to that within the known age groups of WAMRL lobsters. It is difficult to conclude other than that the modes in the lipofuscin concentration–frequency histogram represent annual cohorts of lobsters.

Based on the calibration of the first mode in the lipofuscin concentration–frequency histogram as the 1+ cohort, and confirmation from the known-age material that the intermodal distances represent ~1 year's lipofuscin accumulation, subsequent modes in the histogram represent age-classes 2+ to 6+, with a few potential 7+ individuals also present. Chittleborough and Phillips (1975) reported from tag–recapture studies that a few individuals up to 7 years of age are present on the nursery reefs, but these older age-classes have never been resolved with length–frequency analysis. The present results therefore represent the first frequency analysis to resolve the full range of ages of lobsters present on the inshore nursery reefs. They support the conclusion of migration studies that few, if any, lobsters remain to become permanent residents on the coastal reefs (Phillips 1983).

Each mode in the length–frequency histogram was composed of individuals from a wide range of lipofuscin concentrations. One explanation of this is that it resulted only from random variation in the relationship between lipofuscin concentration and age in what were otherwise temporally cohesive length cohorts. This explanation, however, is not consistent with the very distinct, nonrandom modes in the lipofuscin concentration–frequency histogram. An alternative interpretation of this result is that the length modes do not represent very pure age-classes. Modes assigned 1+ to 4+ were slightly better separated in the length–frequency histogram than in the lipofuscin concentration–frequency histogram. However, if the length–frequency histogram contains hidden

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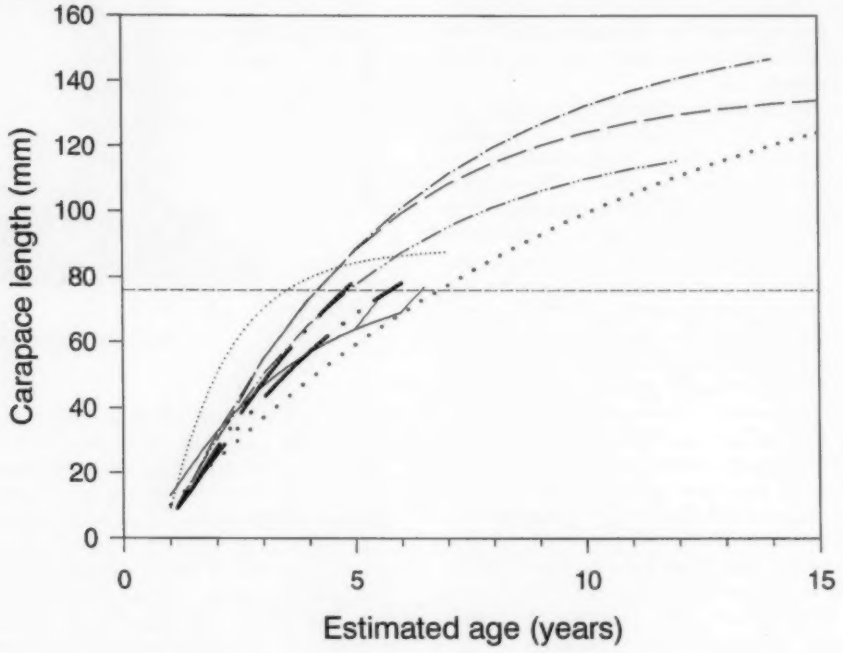
Fig. 6. Preliminary von Bertalanffy curves for length-at-age data from WASE and SMB male (small and large dashed–dotted lines) and female (small and large dashed–double-dotted lines) lobsters from Fig. 4, placed in context with various conventionally derived growth curve segments including those of Chittleborough (1976) for SMB juveniles obtained from length–frequency and tag–recapture data (solid line), Morgan (1980) from moult–frequency and moult–increment data (dashed line), Phillips et al. (1992) from laboratory-rearing and tag–recapture data (large-dotted line), and WAMRL laboratory-reared individuals from the present study (small-dotted line). The horizontal dashed line represents minimum legal size.



older age-classes, as suggested by the lipofuscin concentration–frequency histogram and independent tagging studies, then the fitted length modes are inaccurate and their separation indices are considerably overestimated. If the length modes did not arise from seasonal recruitment pulses, then other explanations for their occurrence are needed. There is a known size gradient of lobsters on the inshore reefs with depth, and Chittleborough (1976) presented evidence from tag–recapture studies that larger individuals not only move to deeper water but grow faster there, where density-dependent resource restrictions may be less (Chittleborough and Phillips 1975). Thus, a lobster's length may be related more to the depth at which it is living than its age. Since the sampling procedure involved collection of lobsters at three different depths, a depth influence on size could explain the predominantly trimodal appearance of the length–frequency histogram and the heterogeneous age composition of these modes. It is apparent that the length distribution is artificially truncated above the 4+ mode due to collection only of lobsters of <80 mm CL. However, the sample would still be expected to contain smaller individuals from age-classes older than 4+. These hidden older age groups appear to be clearly resolved in the lipofuscin concentration–frequency histogram. The present results therefore add to doubt on the usefulness of size–frequency histograms for ageing the lobsters expressed in the literature (Phillips 1990).

The finding that there was no significant ($p > 0.05$) difference in lipofuscin concentrations between males and females after accounting for age differences in the WAMRL lobsters, on its own, needs cautious interpretation due to the small sample size. However, it is consistent with the similarity in modal positions for lipofuscin in male and female SMB lobsters (Fig. 2*b*) and extensive data for other crustaceans (Sheehy et al. 1995*b*, 1996). Therefore, data from both sexes can be pooled to generate a calibration model for estimating age from lipofuscin concentration.

The generalized growth curve is a vital part of the description of the population dynamics of a species and forms part of the yield assessment models for several species of the Palinuridae (Morgan 1980). Inability to age the western rock lobster has, in the past, proved a major problem for determining growth rates in this species. The relationship between CL and lipofuscin-estimated age has proved useful in recent early attempts to estimate generalized growth curves for other species (Sheehy et al. 1995*a*; Belchier et al. 1998). For western rock lobster, these relationships (Fig. 4) show a high degree of individual variation. While part of this is explained by variance in the relationship between lipofuscin concentration and age, wide scatter in length-at-age is considered typical for crustaceans and is well known for the western rock lobster (Chittleborough 1976; Phillips et al. 1992). The lipofuscin-based growth data suggest that any particular size of lobster (apart from the very smallest individuals) can be found in several age-classes and juvenile lobsters from several age groups recruit to the fishery at 76 mm, a conclusion consistent with that of Caputi et al. (1995*a*).

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Fig. 7. (*a*) Length–frequency distribution for SMB sample as in Fig. 2*a* but showing the distribution of lipofuscin-estimated age groups in each length-class. (*b*) Adjusted length – relative frequency distribution for SMB juveniles after weighting the 2+, 3+, and 4+ length modes in the original sample to reflect the average length distribution of lobsters at SMB according to data from Phillips (1990) (poorly sampled 1+ mode omitted). The adjusted distribution of the lipofuscin-estimated age groups is also shown.

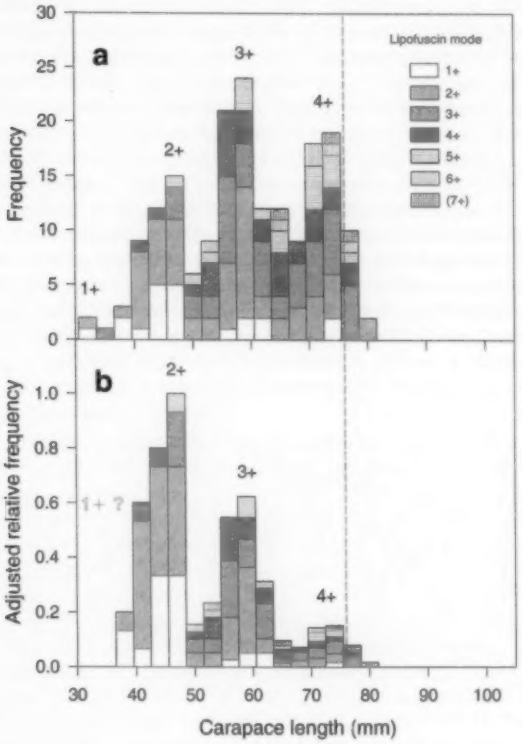
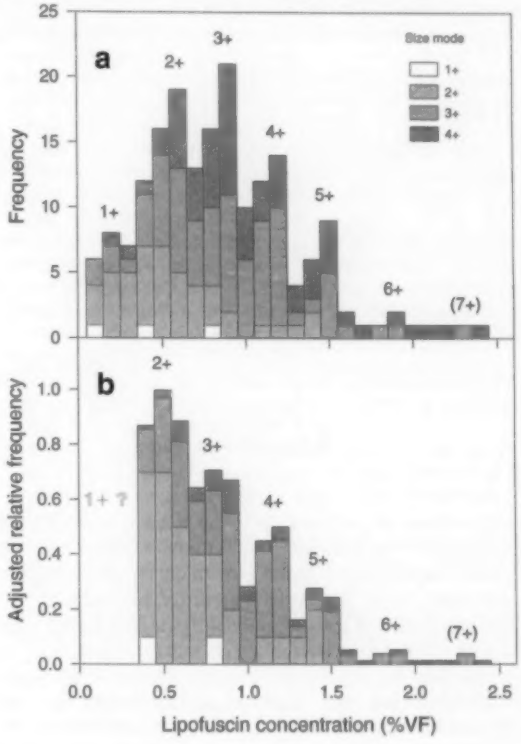


Fig. 8. (*a*) Lipofuscin concentration–frequency distribution for SMB sample as in Fig. 2*b* but showing the distribution of length-estimated age groups in each lipofuscin concentration class. (*b*) Adjusted lipofuscin concentration – relative frequency distribution for SMB juveniles after weighting the 2+, 3+, and 4+ length modes in the original sample to reflect the average length distribution of lobsters at SMB according to data from Phillips (1990) (poorly sampled 1+ mode omitted). The adjusted distribution of the length-estimated age groups is also shown.



Published data on sexual differences in growth rate for western rock lobster appear somewhat contradictory. From tagging experiments, Chittleborough (1976) reported a significant sexual difference in single-moult increments, with juvenile males growing more than females at SMB. He attributed this to the competitive advantage of males under conditions of moderate food shortage. Likewise, Morgan (1977) found that adult males had larger molt increments than females. On the other hand, the growth curves of Phillips et al. (1992) suggested only a very slight difference between males and females. Chittleborough (1976) found no statistically significant differences in the annual growth of juvenile males and females at SMB, and Joll and Phillips (1984) found no significant sexual differences in moult increment of SMB lobsters. It is likely that the reported high level of individual variation obscured differences in growth rate between the sexes in some cases. Preliminary von Bertalanffy models for both the WASE and SMB lobsters in the present study clearly indicated a faster average growth rate for males than for females. Collection of further lipofuscin-based size-at-age information for this long-lived species would allow growth parameters for both sexes under natural conditions to be more accurately determined than has been possible previously using tagging and captive-rearing studies.

Average ages at reaching minimum legal size for SMB males and females suggested by the lipofuscin-based models were 4.8 and 5.8 years, respectively, and 5.3 years (~4.3 years after settlement) for the juveniles taken as a whole. Data for WASE lobsters suggested a slightly earlier recruitment to the fishery, with minimum legal size being reached, on average, 3.5 years after settlement. Mean age at recruitment will vary depending on juvenile growth rates on adjacent nursery reefs; however, when the earlier modal age at which the SMB lobsters reached 76 mm is considered instead of the average age (Figs. 4 and 5), there is very little difference between the SMB and WASE samples. These recruitment ages seem slightly earlier than suggested by the growth curve of Chittleborough (1976) but are in excellent agreement with the established puerulus settlement–catch relationship for the western rock lobster (Morgan et al. 1982; Caputi et al. 1995*a*, 1995*b*) that now forms a reliable model for catch forecasting. The catch, which, under the present conditions of high exploitation,

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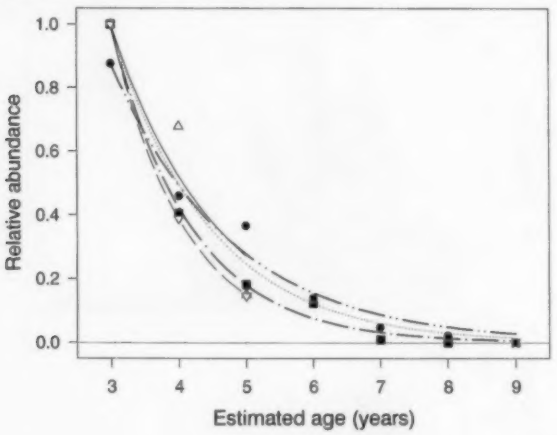
Fig. 9. Relative abundances of male and female SMB lobsters in different lipofuscin-estimated age groups based on the weighted data in Fig. 8*b*. Data for each group are shown relative to the abundance of 3-year-olds in the same group, except those for SMB females which are shown relative to 3-year-old SMB males. Squares, dashed–dotted line: SMB males; circles, dashed–double-dotted line: SMB females; dotted line: SMB all. Data from Phillips (1990), based on length–frequency analyses, are shown for comparison: "estimate 1" (inverted triangles, dashed line) and "estimate 2" (triangles, solid line).



consists mainly of new recruits to the fishery, is most highly correlated with the abundance of settling puerulus 3–4 years previous. The lipofuscin-based growth curves obtained in the present study verify this model by a novel approach.

The abundance of prerecruit juveniles is also highly correlated with catch 1 year later, but a stronger relationship exists for males than for females (Caputi and Brown 1986; Caputi et al. 1995*a*). It has been suggested previously that this could be because the size-classes used to determine age-classes may be more representative of males than of females. The present results tend to resolve this question. They indicate that, on average, females reach legal size later than males and their age at recruitment to the fishery is more variable than for males (Figs. 4 and 5). For any particular settlement event, the subsequent recruitment pulse into the fishery is less cohesive for females than for males. Females persist on the inshore reefs for slightly longer than males from the same cohort. In the present SMB sample, after 3 years of age, females tended to be more abundant than males in the same age groups, and the eight estimated oldest lobsters (6+ to 7+) were all females. The widest disparity in sex ratio on the reef occurred at an age of about 5 years when females outnumbered males by about 2 to 1 (Fig. 9), probably reflecting an advanced movement of the larger males offshore at this age. For older age groups, the sex ratio on the reef tended to equalize again, apparently as remaining females migrated offshore. Further studies are required to determine if these results are typical.

Recent refinements to the catch forecasting model have centered on defining differences in contributions by pueruli and prerecruits to the catch of "red" and "white" lobster phases in the fishery (Caputi et al. 1995*a*). The present results indicate that further improvements to the models could be achieved by focusing on both general differences and individual variation in growth rate and age at recruitment between males and females. The lipofuscin ageing technique would be useful for defining these sexual differences as well as for examining the age distribution of females in the breeding stock where growth has slowed to the point where size is of no value for estimating age.

Several studies have found that growth of lobsters at SMB was slower than that on other coastal reefs (Chittleborough 1976; Joll and Phillips 1984; Edgar 1990; Jernakoff et al. 1994). This may be due to differences in either the quantity or quality of food available at the different locations. The present data, suggesting that WASE lobsters had a faster average growth rate than those at SMB, are consistent with these earlier observations. Notably, preliminary growth curves for the male and female WASE lobsters (Fig. 6) encompass Morgan's (1980) general curve derived from moult–frequency and moult–increment data. All three curves suggest a much faster growth rate than that of Phillips et al. (1992). The latter authors acknowledged that their relatively low estimated growth rates may due to detrimental effects from handling or tagging. The findings of the present study suggest that the lipofuscin ageing technique would be useful for defining apparent regional differences in growth of lobsters, such as in the Abrohlos Island area, for example. Here, females mature at an atypically small size of 60–70 mm CL.

Conventional estimation of total mortality is hindered by difficulties in resolving age-classes in size–frequency histograms (Morgan 1980; Phillips 1990). Age-based procedures involve following the strengths of specific year-classes in temporally sequential length–frequency histograms (Chittleborough 1970; Chittleborough and Phillips 1975; Morgan 1980; Morgan et al. 1982; Phillips 1990). An approximation can also be achieved, as in the present study, by comparison of successive year-class strengths in the one histogram. Loss coefficients, C, for models fitting the relationship between estimated age and relative abundance for SMB lobsters in the present study (0.86 for males, 0.58 for females, 0.70 for all) fell within the wide ranges reported in the literature for total mortality (Chittleborough 1970; Chittleborough and Phillips 1975; Morgan 1980; Morgan et al. 1982; Ford et al. 1988). However, since (*i*) the present study was confined to prerecruit juveniles to compare modes in size–frequency and lipofuscin concentration–frequency histograms, (*ii*) loss of older individuals represents both emigration and mortality, and (*iii*) settlement and loss are variable from year-class to year-class, the coefficients must be treated with appropriate reservation. The main purpose of our loss analysis, therefore, was to demonstrate the future potential usefulness of the lipofuscin technique for calculating total mortality of the fished stock by relatively straightforward comparisons of year-class strengths in lipofuscin concentration–frequency histograms.

When compared with the maximum known life span of western rock lobster (27 years) and the maximum known lipofuscin levels attainable (~7%VF), it is clear that the present known-age data represent only a small portion of the total life history. From this perspective, the relative individual variation in lipofuscin concentrations within age groups was not very different from that for other species that have been examined (Sheehy 1992; Sheehy et al. 1994, 1996; Belchier et al. 1998). Our results from both the known-age lobsters and frequency

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histogram modal analyses clearly indicate that, in this long-lived species, individual variation in lipofuscin concentration precludes accurate ageing of 100% of individuals to the year. However, our results indicate that use of lipofuscin concentration frequencies to resolve annual cohorts offers a substantial improvement over conventional procedures. This is an important finding with broad implications that therefore requires confirmation by further studies. Present indications are that in European lobster, for example, where lipofuscin accumulation rates are slower, a greater longevity is reached and any particular size group is composed of more cohorts than for western rock lobster (Sheehy et al. 1996; M. Sheehy, unpublished data). Consequently, lipofuscin concentration–frequency analysis in these lobsters may require greater sample sizes (France et al. 1991) to resolve cohorts than that used in the present study. As noted recently (Sheehy et al. 1996), more emphasis now needs to be placed on expediting the lipofuscin assay procedure for routine fisheries applications.

Our findings support the original hypothesis that lipofuscin offers a new basic dimension for detection of cohorts in crustacean populations. The technique should permit independent assessment of current population parameter estimates and sexual and regional differences in these parameters for the western rock lobster and other crustaceans.

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Thermal limits and ocean migrations of sockeye salmon (*Oncorhynchus nerka*): long-term consequences of global warming[1]

D.W. Welch, Y. Ishida, and K. Nagasawa

Abstract: Ocean surveys show that extremely sharp thermal boundaries have limited the distribution of sockeye salmon (*Oncorhynchus nerka*) in the Pacific Ocean and adjacent seas over the past 40 years. These limits are expressed as a step function, with the temperature defining the position of the thermal limit varying between months in an annual cycle. The sharpness of the edge, the different temperatures that define the position of the edge in different months of the year, and the subtle variations in temperature with area or decade for a given month probably all occur because temperature-dependent metabolic rates exceed energy intake from feeding over large regions of otherwise acceptable habitat in the North Pacific. At current rates of greenhouse gas emissions, predicted temperature increases under a doubled CO_2 climate are large enough to shift the position of the thermal limits into the Bering Sea by the middle of the next century. Such an increase would potentially exclude sockeye salmon from the entire Pacific Ocean and severely restrict the overall area of the marine environment that would support growth.

Résumé : Les campagnes d'évaluation océaniques révèlent que des limites thermiques très prononcées ont restreint la répartition du saumon rouge (*Oncorhynchus nerka*) dans l'océan Pacifique et dans ses mers bordières au cours des 40 dernières années. Ces limites sont exprimées comme une fonction échelon dont la température qui définit la position de la limite thermique varie mensuellement selon un cycle annuel. La netteté de la différence de température au bord limitrophe, les différentes températures qui définissent la position du bord limitrophe pendant l'année et les subtiles variations de température d'une zone ou une décennie pour un mois précis découlent probablement du fait que les taux métaboliques thermosensibles dépassent l'absorption d'énergie de la prise alimentaire sur de vastes régions de milieu autrement acceptables du Pacifique Nord. Si les émissions de gaz à effet de serre continuent au rythme actuel, les augmentations de température prévues dans un climat où la quantité de CO_2 est doublée seront suffisantes pour causer le déplacement des limites thermiques jusqu'à la mer de Béring d'ici le milieu du siècle prochain. Une telle augmentation pourrait complètement chasser le saumon rouge de l'océan Pacifique et restreindre considérablement les milieux marins propres à la croissance de la population.
[Traduit par la Rédaction]

Introduction

The ocean migration of Pacific salmon is generally believed to involve a counterclockwise movement south in winter and north in summer following the currents of large oceanic gyres (Neave 1964; Royce et al. 1968; Pearcy 1992). Neither temperature nor salinity has been found to be either a strong or consistent determinant of the ocean distribution of Pacific salmon (Burgner 1991), and the influence of oceanographic features on the distribution and migration of sockeye salmon (*Oncorhynchus nerka*) has not been clearly established (Bakkala 1971). The primary oceanographic factor influencing the southern limit to the distribution of Pacific salmon has in the past been variously identified as sea surface temperature (SST) (Ogura and Takagi 1987), subsurface temperatures (Fujii et al. 1965), and various oceanic frontal structures (e.g., Tabata 1983).

The first evidence for strong thermal limits was observed during a spring 1990 survey of Pacific salmon distributions in the Gulf of Alaska (Welch et al. 1995). To further examine possible limits on the distribution of Pacific salmon, a major survey effort involving a total of eleven 1-month surveys was conducted by the Japanese and Canadian governments during the spring, summer, and winter of 1992. These surveys covered all areas of the North Pacific, and a number were repeated in subsequent years. The final survey was a trans-Pacific survey in January 1996 that resurveyed the areas first examined in December 1992 (Fig. 1). These data were combined with historical data from Canadian and U.S. salmon longline surveys for the 1950s and 1960s (Turner and Aro 1968), archived Canadian gillnet surveys from the 1950s and 1960s, modern Canadian data collected in the 1980s and 1990s, and data from all Japanese ocean salmon gillnet surveys completed since 1972.

We report here the limits to the distribution and abundance

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[1] Figures of the differences in thermal limits between areas and decades for November–March, April, May, and September may be purchased from the Depository of Unpublished Data, CISTI, National Research Council of Canada, Ottawa, ON K1A 0S2, Canada.

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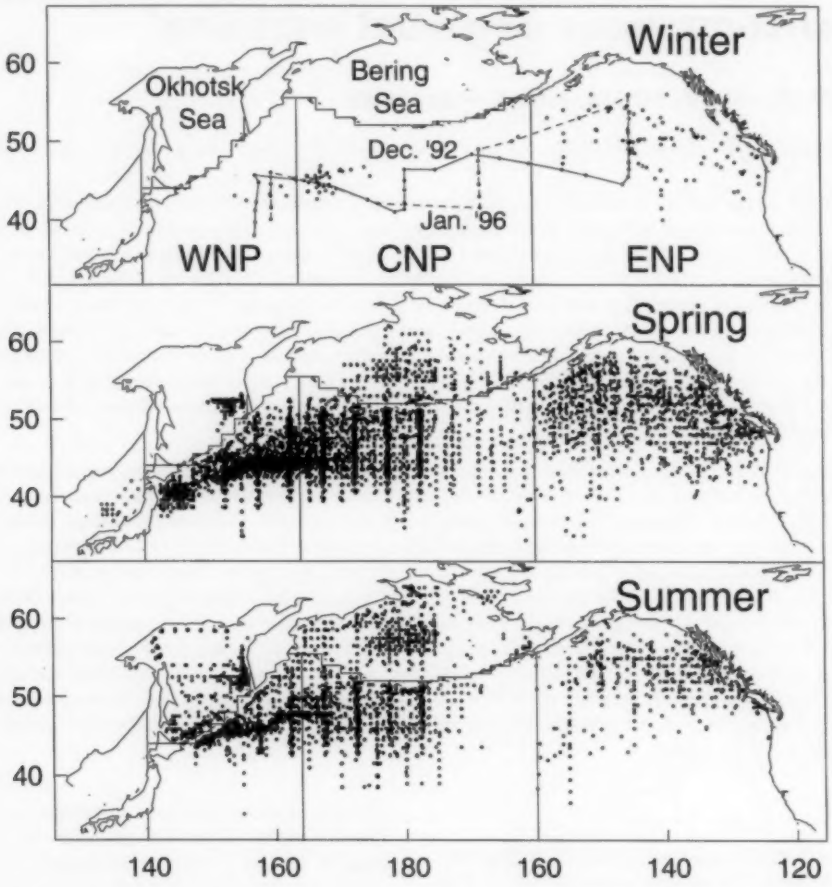
Fig. 1. Distribution of available observations on salmon abundance and temperature, 1956–1996 ($N = 20\,397$) and the geographic divisions of the North Pacific (eastern, ENP; central, CNP; western, WNP) used later. The true density of the data is poorly represented because many stations are overplotted dozens of times. Note that the December 1992 and January 1996 trans-Pacific surveys include observations from all three geographic areas. Winter, November–March; spring, April–June; summer, July–September.



of sockeye salmon using the entire data set ($N = 20\,397$). These data represent a remarkable sampling effort extending over the 40-year period 1956–1996 and covering the entire North Pacific Ocean and adjacent seas. They are the result of a total sampling effort equivalent to 29.1 years of continuous ship survey time, after excluding time lost to weather or in transit. Our results demonstrate that extremely sharp thermal limits to the oceanic distribution of Pacific salmon occur throughout the year. We also show that the temperature defining the thermal edge changes, primarily between months but secondarily between regions or decades examined, probably reflecting the balance between losses due to temperature-dependent basal metabolic processes and energy gained from foraging.

Methods and materials

At each location the catch of each species of salmon was recorded along with the level of fishing effort and a limited number of observations on simultaneously collected oceanographic variables, chiefly SST. Three fishing gears are used in ocean salmon surveys. We defined a unit of fishing effort as being 1 tan (50 m) of gill net, 1 hachi (138 m) of longline, and 1 km of distance towed for surface rope trawls. Catch per unit fishing effort (CPUE) observations were normalized by dividing by annual mean CPUE for each gear type separately to remove interannual variations in abundance and differences between gear types.

The majority of the observations on salmon abundance are based on 8-m-deep gill nets fished at the surface. The remainder of the observations are based on surface longlines (fished in the top 1.5 m of the water column) or surface trawls (0–40 m depth interval). Direct observations of acoustically tracked free-swimming sockeye in the open ocean show that sockeye, as with the other species of Pacific salmon, are strongly surface oriented (Manzer 1964; Ogura and Ishida 1995). Ogura and Ishida (1995) reported that sockeye in the open ocean spent 70% of their time in depths <10 m (mean swimming depth 9.9 m) and maintained an overall vertical distribution almost entirely confined to the upper 40 m of the water column, where nearly isothermal conditions prevail. Deep-diving behaviour was only observed for sockeye salmon (Ogura 1994), and only 1% of the total time was spent below the surface mixed layer by this species during rapid deep dives at near-maximum burst swimming speeds (4.4 body lengths/s). Ascents back to the surface mixed layer were nearly as

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rapid (2.8 body lengths/s), suggesting that this behaviour was probably an adaptation to escape predators and indicating the strong preference for remaining within the surface mixed layer.

Echosounding surveys carried out concurrently with the January 1996 trans-Pacific survey confirmed the strong surface orientation of Pacific salmon (Sakai et al. 1996). Acoustic targets were only identified within the surface mixed layer and at temperatures below the thermal limits observed in winter from the trawl survey (see below). As the mixed layer depth of the subarctic Pacific is >50 m in summer and 80–100 m in winter, the simultaneously collected surface measurements therefore provide a reasonable characterization of the thermal environment of near-surface (<40 m) conditions.

Limits to ocean distributions

The extensive data allow us to subdivide the data by species, month, decade, and geographic region in a set of trellis plots (Cleveland 1993) to examine interactions with oceanographic variables on a consistent set of axes. We concentrate here on analysing the distributional limits for one species, sockeye salmon (*O. nerka*). The sharp thermal limits we report below for sockeye are also evident for the other species of *Oncorhynchus* (which are also caught during the same fishery surveys), but the temperatures defining the upper thermal limits are species specific and generally higher, particularly in the spring and summer. Detailed analysis of the thermal limits for the other species will be reported elsewhere.

In Fig. 2, we plot the logarithm of the catch observations versus SST for all areas combined in the first column, partitioning the data by months. The transformation $\log_e$(CPUE + 1) preserves the zero catches as zero. With the exception of October, when there are no data, an upper thermal bound on the distribution is evident in all months and all regions where sampling extended into sufficiently warm ocean regions. For the "winter" months of November through March, sockeye are confined to regions ≤7°C. Thermal limits increase from April through August, when they reach a maximum of about 15°C. The limit then declines again until finally returning to the 7°C level by November.

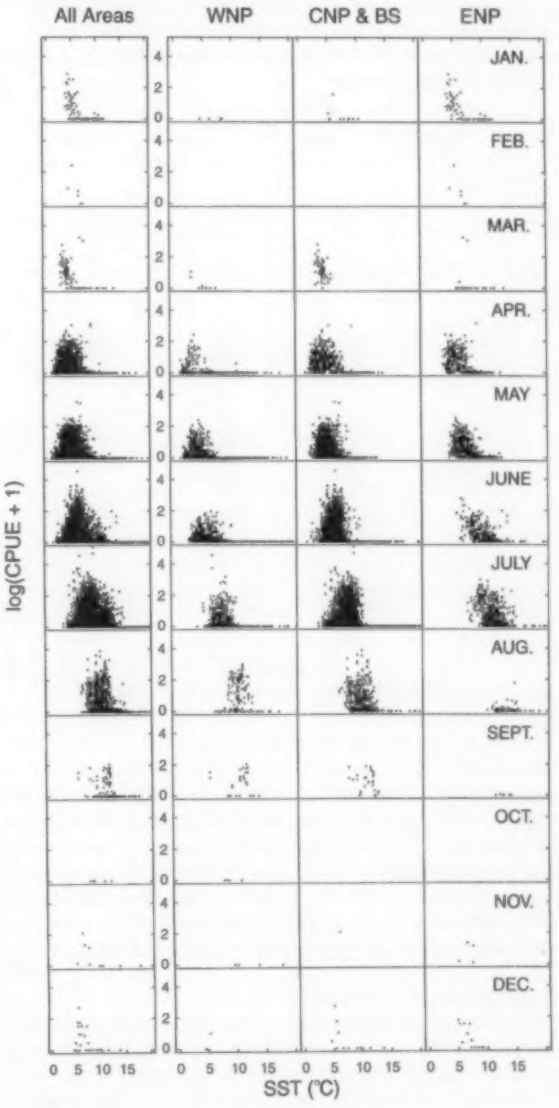
Although the sockeye distribution is clearly bounded to the south by higher temperatures, the thermal limits are not particularly sharp for the aggregated data. Subdividing the data into three regions (Fig. 2, right-hand columns) reveals a much sharper relationship in each month. The highest thermal limits are generally observed in the eastern Pacific, demonstrating that a single uniform response to temperature does not occur.

Further subdivision of the July data by decades and geographic areas shows an extremely sharp response to SST (Fig. 3). The same sharpness is also seen for the other months when similarly subdivided (e.g., Figs. 4 and 5); thus, earlier studies did not identify sharp thermal limits partly because combining data for different months and regions with different thermal limits blurs the response to temperature.

Two of the major limitations of the 40 years of data are the limited number of observations taken in winter or warmer regions where salmon are likely absent and the ambiguity of the available data for some months. For example, sampling always occurred within the sockeye distribution in February (Fig. 2), so the existence, sharpness, and location of the edge are unknown.

The December 1992 and January 1996 trans-Pacific surveys provide a specific opportunity to examine the sharpness

Fig. 2. Monthly comparison of $\log_e$(CPUE + 1) versus temperature. The first column shows the observed response for all areas and decades combined. The next three columns show the monthly changes in thermal limits defining the southern edge of the sockeye distribution for three separate geographic areas of the North Pacific (BS, Bering Sea). The seasonal cycle is similar in all areas, but monthly limits are sharper because they occur at different temperatures in different regions; for this reason, the aggregate response shown in the first column is blurred. A seasonal cycle is clearly evident and far below lethal temperatures (23–25°C; Coutant 1977).



of these thermal limits. The winter distribution of Pacific salmon had previously been described as extending south to about 40°N, to temperatures of about 10–11°C (Manzer et al. 1965). However, we caught no salmon at temperatures above 7.4°C in December 1992 or 7.3°C in January 1996. In

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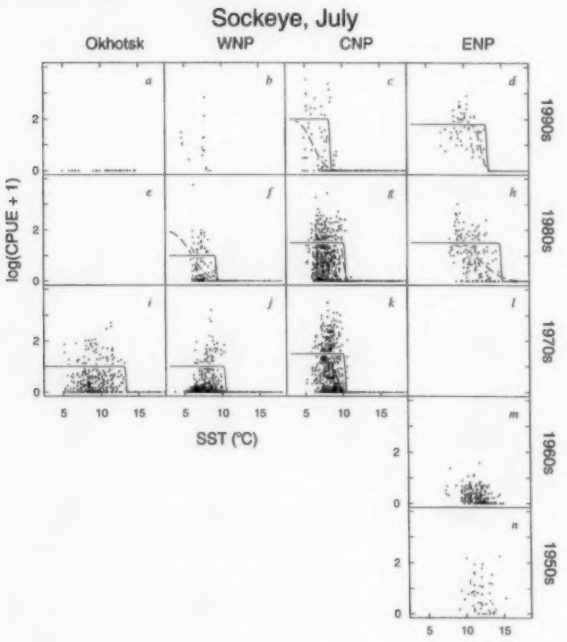
Fig. 3. Differences in thermal limits between areas (columns) and decades (rows) for July, the month with the greatest amount of available data. The thermal limits are now extremely sharply expressed, with abundance dropping to zero in a fraction of a degree Celsius. In three cases (Figs. 3*c*, 3*f*, and 3*h*) the ML estimate described in the text (broken line) indicates that a gradual decline in sockeye density with increasing SST provides the best description of the response, but fails to describe the thermal edge. We refit the model constraining $\sigma_T = 0.1°C$, equivalent to assuming that a sharp step function exists. The estimation problem then reduces to describing the position of the thermal limit. In these three cases the constrained model (solid line) captured the thermal edge; for the other possible comparisons the unconstrained and constrained estimates of the shape of the response are nearly identical. Results for other months are similar.



reexamining the data from the 1950s and 1960s, we found that records of salmon at warmer winter temperatures were primarily limited to a few coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*Oncorhynchus mykiss*), which were caught at temperatures up to 10–11°C, near the subarctic boundary at 40°N. However, a clear upper thermal limit on the midwinter distribution of all species of salmon except coho was evident.

Generalized additive models

Lethal limits for Pacific salmon are generally well above 20°C (Coutant 1977), so the remarkably low thermal limits observed result in sockeye being excluded from vast areas of the North Pacific that are otherwise potentially habitable. The sharpness of the edge that results when the data for different months, areas, and decades are considered separately suggests that the primary response is often nearly a step function, with sockeye expressing strong avoidance behaviour within a relatively narrow range of temperatures, and temperature having relatively little influence on distribution below this threshold.

A step function implies that temperature has no influence on behaviour except at the limit, where essentially all individuals must simultaneously express strong avoidance behaviour. The surprising nature of this response has important implications for understanding why sockeye avoid exceeding these temperatures during their ocean migrations. We used a generalized additive model (GAM) to relate the distribution of sockeye to temperature, expressing the abundance as the logarithm of the number of individuals caught at the ith station per unit sampling effort, $\ln(n_i + 1)$. Specifically, the GAM model we used can be expressed as

$$(1) \qquad \ln(n_i + 1) = \mu + f_T(T_i) + \varepsilon_i$$

where μ represents the average abundance, $f_T(T_i)$ represents the loess smooth of temperature, and ε_i represents the error distribution for the ith observation.

GAMs were estimated assuming a negative binomial (NB) error distribution with known "shape parameter" (Venables and Ripley 1994). The shape parameter is equivalent to the degrees of freedom (df), k, on which each observation is based (Welch and Ishida 1993). We modified the definition of the NB family within the statistical analysis program S-Plus to allow the specification of a different number of df k_i for each observation, using $k_i = N_i/15$, where N_i is the number of metres of gill net or longline used, or distance trawled, at the ith station. As the sampling effort or df approaches infinity, the NB distribution approaches the Poisson. Thus, for finite df, the distribution we used has heavier tails and higher variance than the Poisson.

In GAMs, locally weighted fitting is used to calculate a nonparametric estimate of the relationship between sockeye abundance and potential explanatory variables using a realistic model of the observational error (Hastie 1992). In essence, the GAM method is the maximum likelihood equivalent of nonparametric smoothing techniques. The advantage of GAMs is that they do not require initial specification of the form of the functional relationship between SST and salmon abundance, and therefore permit a realistic evaluation of the appropriate form of the relationship that should be specified in our more detailed analyses (see below).

In our analysis, we used a loess curve with $\lambda = 1$ and generally chose a data window as narrow as possible in calculating the local response of sockeye abundance to SST, typically using 10–20% of the data points to measure the average response. The sharpness of the response is therefore limited by the smoothing specified; use of a narrower data window results in a more sharply defined response but greater statistical uncertainty in the estimated mean response. The observations on the ocean distribution of salmon are typically spread over a 15°C range of temperatures, with most observations at lower temperatures, so the transition width for even a perfect step function will be spread over roughly 3–6°C, depending on the exact width of the smoothing window chosen. The GAM analysis therefore permits an assessment of whether the change in sockeye abundance is "step-like," with the major drop in abundance occurring in 3°C or less.

Applying the GAM analysis to the sockeye data indicates that abundance is related to ambient temperature as a threshold in both winter and summer, with little measurable effect of SST on abundance away from the edge (Fig. 6). Because the GAM estimate of salmon abundance decreases with increasing

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Fig. 4. Differences in thermal limits for sockeye between areas (columns) and decades (rows) for June. Note that although the thermal limits are sharply expressed, the temperature at which the thermal limit occurs is lower than that evident in July. The fitted curve has been excluded for clarity.

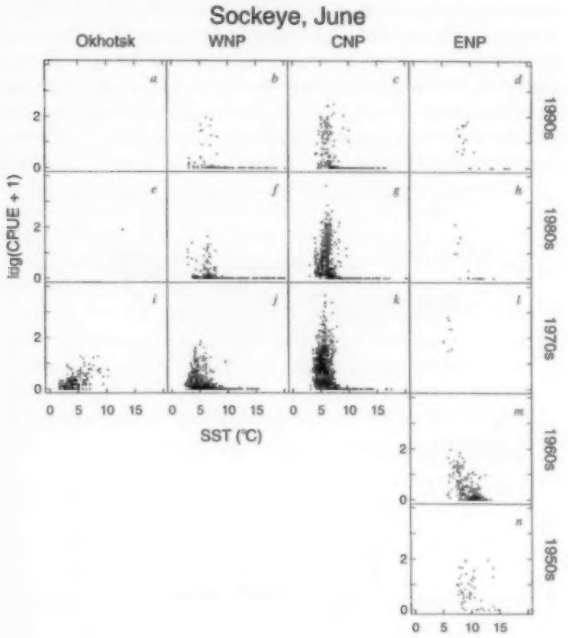
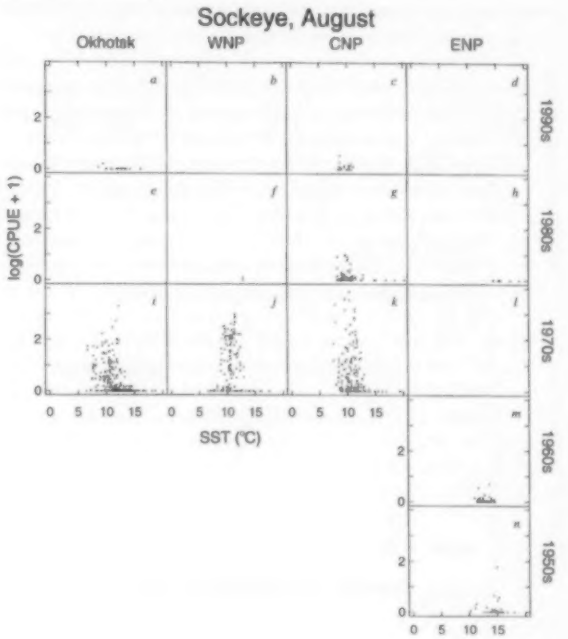


Fig. 5. Differences in thermal limits for sockeye between areas (columns) and decades (rows) for August. Note that although the thermal limits are sharply expressed, the thermal limit occurs at higher temperatures than in June or July. The fitted curve has been excluded for clarity.



temperature over a 2–3°C interval, it is unclear from this analysis alone whether abundance primarily decreases over this relatively narrow thermal interval, or even more sharply than the GAM analysis is capable of resolving (i.e., an extremely sharp step-function response to temperature). However, the analysis does make clear that the primary response to temperature does occur near the thermal limit in a 2–3°C interval and that relatively little change in abundance is evident far from the limit, particularly when sufficient data exist so that the analysis can be performed for a single month and a specific region of the North Pacific. As the maximum likelihood (ML) analysis in the following section makes clear, the response in most months and areas is a very sharp decline in abundance that takes place over only 1–2°C.

Influence of other oceanographic variables

Introducing salinity into the GAM analysis revealed no measurable influence, but the majority of the survey observations do not include information on any oceanographic variables other than SST. Using the World Ocean Atlas (WOA) database of oceanographic observations for the North Pacific (NODC 1994) from 40 to 60°N, we compared the relationship between 10-m SSTs with other simultaneously collected oceanographic variables. After splitting the WOA data into areas, months, and decades to match the previous analyses, we found no strongly coherent relationships between temperature and salinity, O_2, or major nutrients (nitrate, phosphate, silicate) (Fig. 7). The sharpness of the step-function relationship between temperature and sockeye abundance and the absence of a strong relationship between SST and other oceanographic properties therefore strongly suggest that other factors do not play a substantial role in determining the position of the southern edge of the distribution.

Maximum likelihood (ML) analysis

To more precisely measure the influence of temperature on the southern limit, we fit a parametric model that assumes an asymptotic level of sockeye abundance away from the thermal edge and allows estimating T_{crit} and σ_T, the critical temperature and rate of change in abundance near the critical temperature, respectively, by maximum liklihood (Appendix). We concentrate here on defining the variations in the upper thermal limit for different months and areas, since there is little evidence of a lower thermal limit for sockeye.

In most cases, fits of the parametric model provided a realistic description of the sharp thermal limit evident in the data. In three of the possible comparisons for July (Figs. 3*c*, 3*f*, and 3*h*) the ML estimate of the average response of abundance to SST indicated that abundance decreased steadily with SST over the entire range of temperatures. However, in these cases the fits failed to describe the visually evident edge. We therefore refit the model after constraining $\sigma_T = 0.1$°C, which is equivalent to assuming that a sharp edge to the distribution exists, and then estimating the temperature at which the edge is located. The constrained fits were identical to the unconstrained fits in most cases and provided a more realistic estimate of the position of the thermal limit in the other three cases.

Figure 8 summarizes the monthly changes in critical

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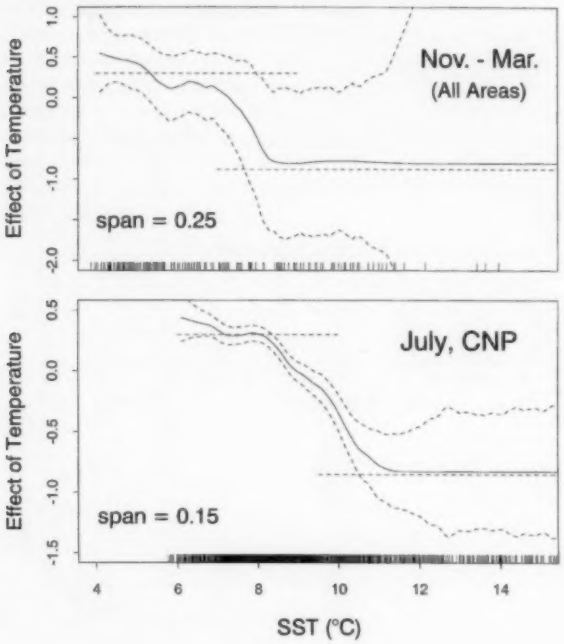
Fig. 6. GAM analysis of the estimated response of sockeye abundance to temperature in winter (November–March) and midsummer (July) and associated 95% confidence interval. Over a wide range of temperatures the estimated response is flat, indicating little influence of temperature on abundance within the distribution. Broken horizontal lines are included for reference.

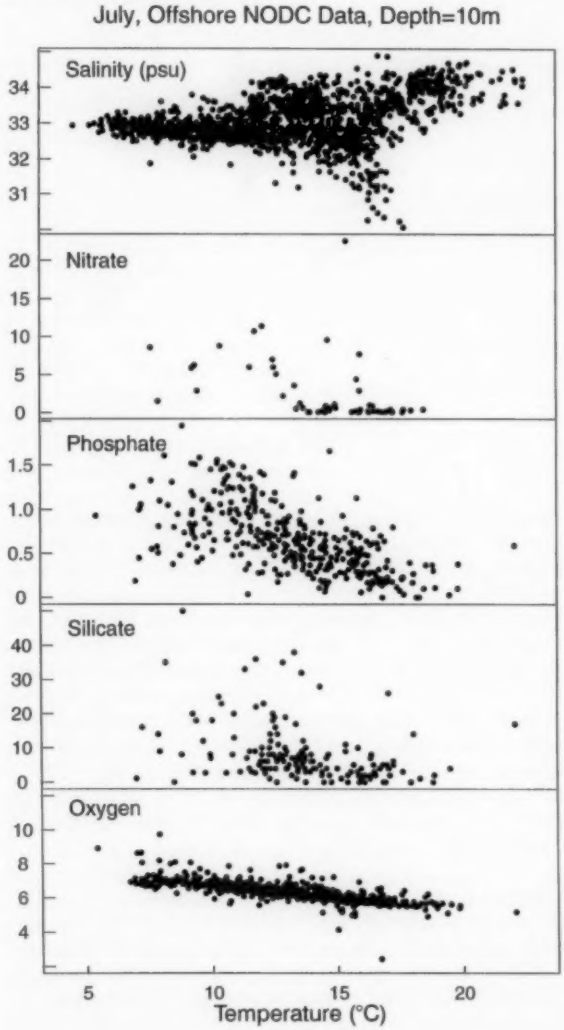


temperatures defining the thermal limits for three areas of the North Pacific after aggregating the data for all decades, and the temperature interval over which sockeye abundance drops by one order of magnitude. Despite the combination of data from different decades with slightly different thermal limits (see below), in most months and areas the thermal limits are sharply expressed, with abundance estimated to decline by an order of magnitude in ≤1°C in eight of 19 possible comparisons. Repeating the analysis after constraining $\sigma_T = 0.1$°C resulted in an almost identical pattern of change, with the thermal limit in the eastern North Pacific consistently found at a higher temperature than in the central or western North Pacific.

Depending on area, the temperature defining the position of the thermal limit varies cyclically from 7°C in winter months to about 13–15°C by late summer. This response is in direct contrast with past theories of salmon migration that suggested a southward movement in winter, but without a strong influence of temperature on distribution (cf. review by Burgner 1991). Within a given month, the thermal limits are also found at different temperatures in different areas of the Pacific; thus, previous studies overlooked the sharp limits because the aggregation of data for different months or regions with different thermal limits results in a blurred and less clearcut response to temperature.

Two alternative explanations for the existence of the extremely sharp thermal limits evident in Figs. 3–5 seem initially possible, but are inconsistent with key aspects of the data. First,

Fig. 7. Comparison of measured values of salinity, nitrate, phosphate, silicate, and O_2 with simultaneous measurements of ocean temperature in July (all data are for observations at 10 m depth). A similar lack of a consistent relationship with temperature is evident for the other months of the year (not shown). The sharp drop in sockeye abundance at the thermal boundaries shown in Fig. 3 makes it unlikely that the sockeye response is actually determined by these oceanographic variables because the observed level of correlation with temperature is insufficient to cause such a sharp drop. Measurements were based on all historical bottle data collected for offshore regions of the Pacific Ocean north of 40°N since 1932 (NODC 1994).



sockeye may be orienting relative to the distribution of prey or predators, which are responding directly to temperature. However, the observed thermal limits differ between salmon species, and Pacific salmon have some trophic overlap and similar predators, so this explanation is unlikely.

A second possibility is that the sharp edge to the distribution

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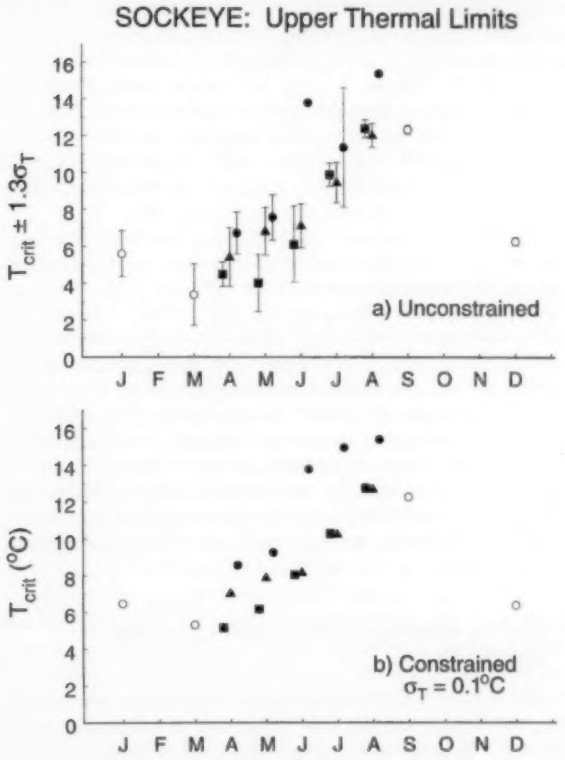
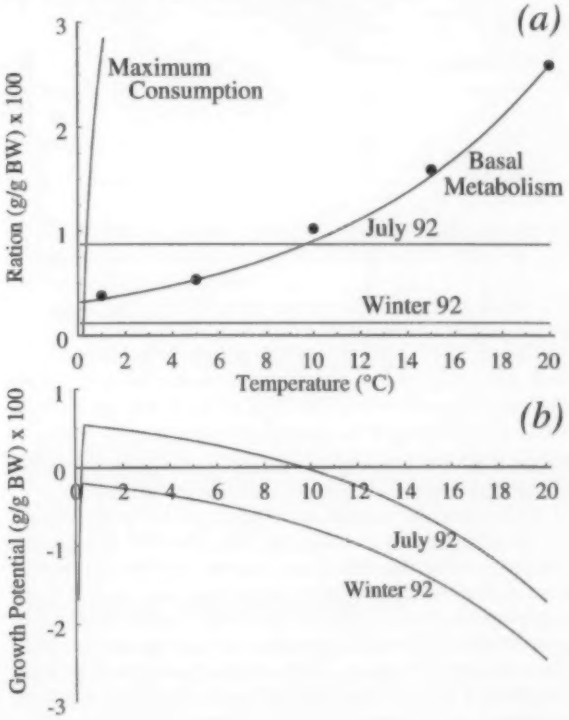
Fig. 8. Estimated critical temperatures defining the southern limit to the sockeye distribution, T_{crit}, in different months. (*a*) Unconstrained. (*b*) Constrained. Squares, WNP; triangles, CNP; solid circles, ENP; open circles, areas combined. The vertical bars in Fig. 8*a* represent the thermal range over which sockeye density drops by a factor of 10, $T_{crit} \pm 1.3\sigma_T$, not the statistical uncertainty in ML estimates of the critical temperature (differences in estimated thermal limits between months for a given region were statistically significant in all cases). In cases where no vertical bars are evident, the estimated thermal range is smaller than the size of the symbol. Figure 8*b* shows the constrained estimates of the monthly thermal limit after fixing $\sigma_T = 0.1°C$. For both Figs. 8*a* and 8*b*, thermal limits increase into the late summer and then decline; in months where sufficient data exist to permit separate estimation of area-specific thermal limits, the eastern North Pacific has significantly higher thermal limits for any given month. The only substantial difference between the results for the constrained and unconstrained estimations is for the eastern North Pacific in July, where the unconstrained estimation gave a substantially lower thermal limit and indicated a very gradual decline in abundance with increasing SST; the constrained estimate is more consistent with the data and the pattern seen for the other months (see Fig. 3).



is geographically fixed. Seasonal warming and cooling of the ocean could then cause the isotherms to move past the edge of the distribution in a predictable and cyclic fashion. However, despite the sharpness of the edge in all months, the geographic position is not fixed, with the greatest southern extent of the sockeye distribution occurring in the spring and very early summer (June) and the winter and summer thermal limits lying several hundred kilometres to the north.

Fig. 9. (*a*) Comparison of laboratory data on temperature effects with seasonally averaged sockeye stomach contents for the eastern North Pacific. Maximum consumption and basal metabolic rates are fitted curves of daily rates (% of body weight (BW)/day) for fingerling sockeye raised in freshwater and fed a high-energy artificial diet; symbols show the original measurements by Brett et al. (1969). Stomach contents are instantaneous observations (% of BW) for much larger ocean-caught sockeye in February, July, and December 1992. The vertical positions of the winter and summer feeding measurements are not directly comparable with metabolic rates because they are not corrected for digestion or repeated feeding throughout the day or possible metabolic differences between the two groups, but provide a conceptual guide to the balance between consumption and metabolism that may occur. (*b*) Potential growth, defined as the difference between food consumed and basal metabolic losses, declines to zero with increasing temperature. Both the maximum and the zero point shift to higher temperatures as food availability increases, but only orientation to avoid negative growth rates (i.e., starvation) is consistent with a step-function response to temperature. The distributional data are therefore most consistent with salmon avoiding thermal regions that result in starvation, i.e., regions where energy lost to metabolism exceeds the energy gained from foraging. The exact positions of the zero crossings in different seasons are dependent on daily rates of consumption and activity costs in the ocean, which have not been measured.



Bioenergetic control

The extreme sharpness of the thermal limits indicates that on evolutionary time scales the development of a strong behavioural response to temperature is very important to overall

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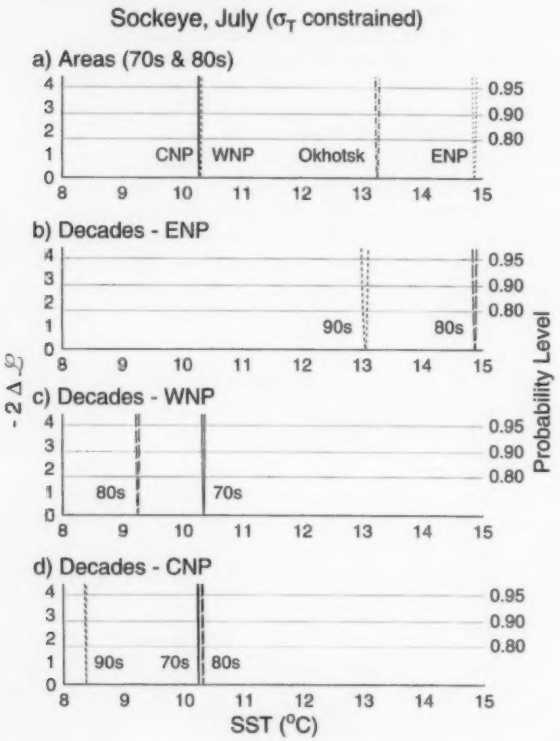
Fig. 10. Likelihood profiles comparing the differences in the estimated critical temperature defining the upper thermal limit for July between (*a*) areas and decades for (*b*) ENP, (*c*) WNP, and (*d*) CNP. Profile values show the relative likelihood that different temperatures define the actual position of the thermal limit, calculated as twice the difference in log-likelihoods from the ML estimate of T_{crit}, which occurs at $-2\Delta\mathcal{L} = 0$. The ML estimate of the critical temperature occurs where the likelihood profile touches the *x*-axis. Probability levels (right axis) are based on a $-2\Delta\chi^2_{1\,df}$ distribution, appropriate for testing differences in one parameter. For consistency, all critical temperatures were calculated using the constrained ML model assuming $\sigma_T = 0.1°C$. Results for the unconstrained model are similar.



fitness. The observed response also raises the question of what mechanism underlies this thermal avoidance behaviour.

Basal metabolic rates increase exponentially with temperature in cold-blooded animals (Fig. 9*a*), resulting in a growth maximum when the difference between maximum potential rates of food intake and basal metabolism plus activity costs is greatest (Brett et al. 1969). In laboratory studies, fish growth is maximized at lower temperatures as food is reduced. Fish, given the opportunity to choose, move to the environmental temperature yielding maximum growth when food is limited (Beitinger and Fitzpatrick 1979; Jobling 1981; Mac 1985). However, although qualitatively correct in predicting a migration to colder waters in times of reduced food availability, growth declines roughly symmetrically about the growth maximum (Weatherley and Gill 1995), so either a decrease or increase in temperature should have similar effects.

A step-function response to temperature is inconsistent with a strategy of growth maximization. This response is, however, consistent with behaviour where thermal avoidance is only expressed once metabolic losses due to increased temperature exceed the energy gained by feeding (Fig. 9*b*). Sockeye can maintain positive growth rates at higher ocean temperatures as food levels seasonally increase, consistent with the observed expansion into warmer waters in spring and summer. The observed distributional limits therefore likely reflect the boundary where growth rates become negative if exceeded, and starvation sets in even in the presence of significant levels of prey.

Although information on the relative abundance of lower trophic level prey is limited, a comparison of the position of the thermal limits shown in Figs. 3–5 and 8 generally supports the expectation of higher thermal limits in regions or decades with greater productivity. Thermal limits in July were 3–5°C higher in the Okhotsk Sea and eastern North Pacific (Fig. 10*a*), areas with enhanced nutrient levels due to estuarine recirculation and enhanced upwelling (Tully and Barber 1960). These areas presumably have correspondingly higher standing stocks of zooplankton than the central and western North Pacific.

Both zooplankton and salmon abundances approximately doubled between the 1960s and 1980s (Brodeur et al. 1996) in the eastern North Pacific. Unfortunately, little sampling effort was expended south of the thermal limit in the 1950s and 1960s (Figs. 3*m*, 3*n*, 4*m*, 4*n*, 5*m*, and 5*n*), so the distributional data are uninformative about the existence and position of a thermal limit. Only with the inclusion of data from the directed Canadian and Japanese cruises of the 1990s, which focussed much effort near this boundary, does the characterization of the thermal edge become substantially better (Fig. 10*b*).

A testable prediction for all regions of the North Pacific is that the ratio of zooplankton to salmon abundance fell following the 1977 (Beamish and Bouillon 1993; Hayward 1997) and 1990 climate shifts (Trenberth and Hoar 1996), since thermal limits were clearly lower in recent decades (Figs. 10*b*, 10*c*, and 10*d*). Unfortunately, the plankton data necessary to test this prediction are not readily available. However, taken as a whole, our results are consistent with the prediction that the highest thermal limits should be observed in those months, areas, and decades with the highest food levels. Productivity changes therefore shift the temperatures defining the thermal limits in predictable ways, with the greatest differences occurring between months and the finest scale differences between decades within a given month and region.

Climate change and ocean carrying capacity

Models of the world's climate predict significant warming in the next century as the CO_2 content of the atmosphere increases. The greatest warming is expected in winter and high latitudes (Watson et al. 1995). The Canadian Climate Centre's equilibrium climate model couples a simplified 50-m surface mixed layer representation of the ocean to a multilevel atmosphere and provides a midrange prediction of the amount of climate warming expected under a doubled CO_2 climate (Boer et al. 1992; McFarlane et al. 1992). Applying the predicted mean position of the isotherms limiting the seasonal distribution of sockeye under current and future climates provides a forecast of their possible future ocean distribution.

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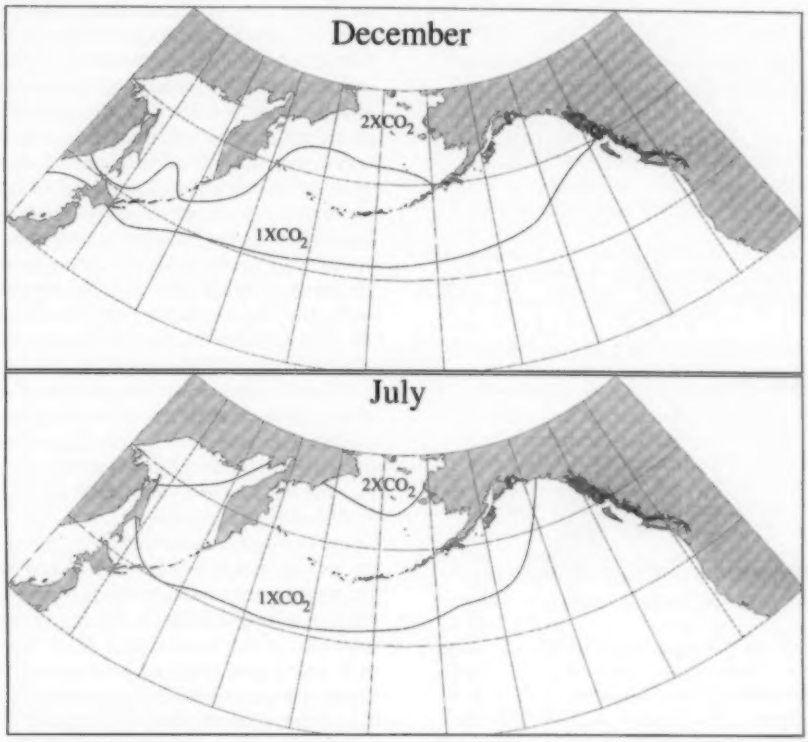
Fig. 11. Comparison of the predicted winter (7°C) and summer (12°C) positions of the sockeye salmon distribution under current and future climates (Albers equal area projection). Under a doubling of atmospheric CO_2 the area of acceptable thermal habitat in the North Pacific is predicted to decrease to zero in summer and decline sharply in winter. The predictions are based on the Canadian Climate Centre's coupled ocean–atmosphere general climate model (Boer et al. 1992; McFarlane et al. 1992).



Regions of thermally acceptable habitat are predicted to shrink dramatically under a doubled CO_2 climate, largely restricting the distribution to marginal seas in northern regions and presumably increasing the density of sockeye in the area remaining (Fig. 11). Thus, although much attention has been paid to the possibility that some stocks of salmon near the southern end of their distribution may be adversely affected by climate warming in freshwater (e.g., Francis and Sibley 1991; Levy 1992), events happening in the marine phase could be even more disruptive.

If the surface mixed layer does warm past currently maintained thermal limits, sockeye would appear to have three choices: (*i*) develop the ability to migrate into the Bering Sea (and return), (*ii*) begin to vertically migrate to stay below the thermocline for much of the day, migrating into surface waters to opportunistically forage for short periods of time, or (*iii*) incur the energetic or other penalties associated with thermal environments that they now strongly avoid.

Young sockeye are observed to vertically migrate in thermally stratified lakes prior to migration to the ocean when surface waters become very warm (Henderson et al. 1992), and adult chum salmon (*Oncorhynchus keta*) have been observed to migrate at depth to avoid the high temperatures of the Tsugaru current off Japan (Ueno 1992). However, as we have noted, there is excellent evidence that Pacific salmon are strongly surface oriented, so although the innate behaviour needed to permit vertical migration appears to exist, the costs associated with expressing new behaviour and avoiding the surface mixed layer are likely significant.

Unless prey abundances increase, the opposite of current predictions (Hsieh and Boer 1992; Hinch et al. 1995), sockeye remaining in the North Pacific may therefore incur significant energy deficits, reducing growth and consequently both their productivity and resulting population sizes. Irrespective of the degree of warming, the combination of decreasing prey abundance and shrinking thermal habitat due to climate warming will likely have negative effects on survival, growth, and size- and age-at-maturity that will reduce future productivity (Forbes and Peterman 1995).

Comparison with prior studies

A striking aspect of our findings is how sharp the thermal limits are when divided by months, regions, and decades. This suggests that salmon respond with extreme sensitivity to even slight changes in their thermal environment. However, the response evident in our analysis has not been previously noted, raising the question of why our extensive data and methods of analysis show such widespread evidence for sharp thermal limits when most earlier studies indicated a more ambiguous influence of temperature on the distribution of Pacific salmon (cf. review by Burgner 1991).

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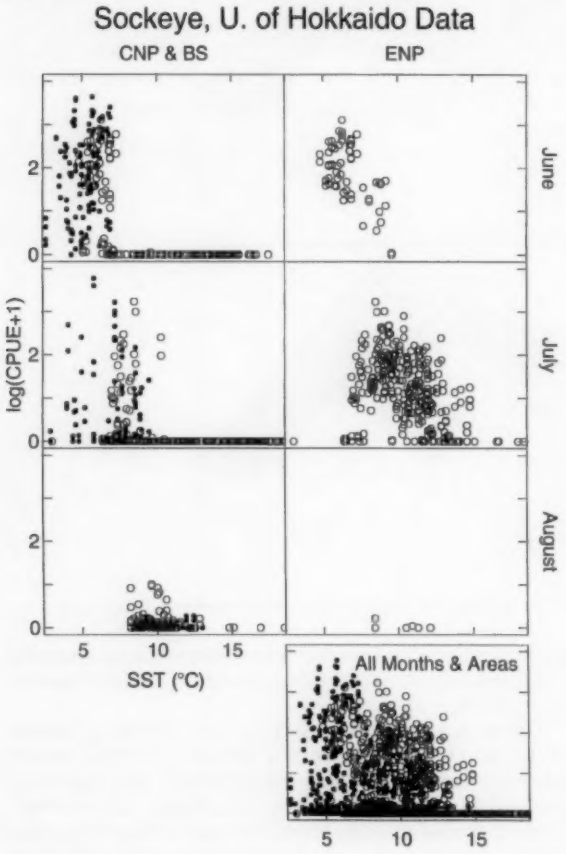
Fig. 12. Comparison of the sockeye response to SST for the University of Hokkaido data (1972–1993) divided by areas (columns) and months (rows) (the 1978–1993 subset is shown using open circles and earlier years using solid circles). The same sharp thermal limits shown in Figs. 3–5 are evident for this subset. Note that, as for the full data set, the aggregation of the data for all months and areas results in a more ambiguous relationship because combining data with differing thermal boundaries blurs the overall response.



A recent elegant and thorough statistical analysis by Pearcy et al. (1996) provides an excellent example. Pearcy et al. (1996) studied the relationship between the abundance of fish and squid measured by surface gill nets using the University of Hokkaido research vessels *Oshoro Maru* and *Hokusei Maru* and a range of simultaneously collected oceanographic data, including SST. As the authors noted, theirs "...is a unique study because it utilizes collections repeated every year for over a decade and a half, over a large region". The observations used in the Pearcy et al. (1996) paper are from 1978–1993 ($N = 513$ samples). The data forming the basis for our paper also contain the same data from Hokkaido University, but for a wider range of years, 1972–1993, and also include data for the Bering Sea ($N = 1924$ samples). Thus the Pearcy et al. (1996) analysis was based on 2.5% of the data used in the current paper. (The full Hokkaido University data set forms <10% of the total used here.)

Pearcy et al. (1996) reported that "...distinct boundaries between species groups and sample groups were lacking" but that "Interannual fluctuations...were most closely related with changes in SST," although "All the physical factors, and latitude, were very highly intercorrelated." These authors did not identify clear thermal limits on the distribution of salmon, but did demonstrate the existence of a northern (chiefly salmon) and southern group of species, thus explaining part of the reason for the association with temperature. They also described a number of other important aspects to the community structure of the North Pacific fauna and noted that the boundary between the species groups moved north or southward by several degrees of latitude in different years, equivalent to a spatial scale of several hundred kilometres.

Analysis of the sockeye salmon response to SST for the University of Hokkaido subset of our database shows that the same sharp thermal limits found for the entire 40-year database are also evident for this more restricted sample (Fig. 12). Pearcy et al.'s (1996) analysis of the aggregated data did not identify the presence of sharp thermal limits in this data set primarily because different temperatures define the thermal edge in June, July, and August. Unless the data are partitioned, the sharpness of the overall response is less clear, essentially because the data set is now a set of several overlapping step functions buried in noise.

Previous difficulties hampering the earlier identification of the thermal limits we report here by a wide range of investigations that used subsets of the same data (e.g., Fujii et al. 1965; Tabata 1983; Ogura and Takagi 1987) can therefore be attributed to two factors. First, there is a need to partition the data into aggregates (i.e., seasons, regions, or periods) with similar thermal limits to prevent the blurring or smearing of the response when data representing different thermal responses are aggregated. Second, standard statistical methods lead to inherent limitations in the analysis that need to be recognized. These methods implicitly require that a smooth and continuous relationship between abundance and temperature exists, rather than the discontinuous response that we have shown characterizes the data. The calculation of a correlation between abundance and SST, no matter how the data are partitioned, will only measure the linear contribution to the overall relationship between two variables. In the case of a step-function response to SST, this contribution can be quite small.

Ecological implications

As greenhouse gases increase and new climates develop, major losses in ocean habitat are likely to occur for all salmon species. Large areas of the North Pacific may warm past current thermal limits for much of the year, reducing ocean habitat. However, perhaps the most surprising prediction is that none of the Pacific Ocean may lie within the thermal limits that have sharply defined the distribution of sockeye salmon over the last 40 years.

In British Columbia alone, there are more than 9600 genetically separate stocks of Pacific salmon (Slaney et al. 1996). British Columbia formerly produced about 15% of Pacific salmon production, making for a rough estimate of >60 000 genetically separate populations in the North Pacific. Because salmon abundances drop so sharply with temperature, most

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populations of each species must be responding identically; otherwise, the sharpness of the response would be blurred. Whatever the specific causes, thermal influences on the long-term survival of each species must be very important, or such a uniform response to temperature would not be observed.

The sharpness of the boundaries that we have found in all months suggests that Pacific salmon evolved acute thermal sensitivities because factors influencing growth in the ocean have profound effects on survival over evolutionary time scales. The boundaries also imply that food is limiting growth in the ocean over virtually the entire year because thermal limits should otherwise be less sharply expressed and seasonally unchanging. The clear response seen consistently in all partitions of our data also raises the question of whether many other cold-blooded organisms may also express similar distributional responses to temperature that have so far gone unrecognized, for similar reasons.

The expression of strong avoidance behaviour over the range of temperatures observed might seem consistent with the large body of evidence that demonstrates temperature selection based on *optimizing growth* rather than avoiding growth deficit, as we had earlier suggested (Welch et al 1995). However, close examination of published growth curves (e.g., Shuter and Post 1990, fig. 3; Weatherley and Gill 1995) shows that the response of growth rate to temperature in fish tends to be relatively flat-topped and symmetrical near the optimum temperature. Neither property is consistent with an abrupt decline in abundance over a very narrow temperature range and relatively little change in salmon abundance away from the thermal limit.

In summary, the sharp ocean-wide response to temperature that we have demonstrated restricts the distribution of sockeye salmon to a relatively small area of the subarctic Pacific for much of the year. However, it is the strength of the response and the remarkably low temperatures at which it occurs that are of greater concern. With climate change, large areas of the North Pacific may be unable to support growth. Ironically, because the vast majority of salmon research has focussed on the freshwater phase of the life history (e.g., review by Magnuson et al. 1996), even the complete shift of open-ocean salmon distributions into the Bering and Okhotsk seas could be missed without ocean research to complement freshwater programs, and the consequent losses in productivity incorrectly attributed to freshwater habitat changes.

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Appendix

The southernmost edge of the salmon distribution can be defined by assuming an average critical temperature at which animals express avoidance behaviour, T_{crit}, and Gaussian variability between individual animals in the temperature at which this behaviour occurs, σ_T (Welch et al. 1995). Although the response of individual animals cannot be measured from the fisheries surveys, the population-level response is the proportion of the initial density of animals in the interior of the distribution, μ, that still remains at some temperature, T_i, or

$$(A.1)\quad n(T_i) = \mu(1 - \Phi(T_i|T_{crit}, \sigma_T))$$

where $\Phi(T_i|T_{crit}, \sigma_T)$ defines the cumulative Gaussian probability (or proportion) of all individuals that have responded by some temperature T_i. Estimates of the midpoint, T_{crit}, and the rate of change in abundance with temperature around this point, σ_T, define the rate at which abundance declines with increasing temperature at the thermal limit (Welch et al. 1995).

ML estimates of the mean density within the distribution, the critical temperature defining the edge, and the rate of decrease in abundance with SST at the edge, (μ, T_{crit}, σ_T), were obtained by minimizing the negative log-likelihood of the data with respect to the parameters, assuming that errors in the observations of catch were distributed negative binomially (Welch and Ishida 1993; Welch et al. 1995). Likelihood profiles of the response of sockeye abundance to SST allow us to objectively examine the effect of seasonal, regional, and decadal differences in the temperature defining the thermal limits. Profiles were calculated by taking twice the difference in log-likelihoods from the ML estimates for various alternative temperatures T_{alt} (allowing all other parameters to vary freely) and finding the relative likelihood

$$-2\Delta\mathcal{L} = -2[\mathcal{L}(T_{alt}, \sigma_T^*, \mu^*) - \mathcal{L}(T_{crit}^*, \sigma_T^*, \mu^*)].$$

Confidence intervals on estimates of T_{crit} can be calculated by setting $-2\Delta\mathcal{L}$ equal to a specific value from a $\chi^2_{1,1-\alpha}$ distribution. This is a large-sample test appropriate for testing differences in a single parameter.

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Model complexity and catch-age analysis

Laura J. Richards and Jon T. Schnute

Abstract: Catch-age analysis has become a standard tool of fisheries stock assessment. Modern catch-age models can contain dozens or even hundreds of parameters to be estimated. In this paper, we compare the performance of a typically complex catch-age model with a simpler model in which the population age structure is represented only by the annual mean age. We find that the population trajectory estimated from the simple model is in general agreement with the trajectory from the catch-age analysis. We suggest that the simple model can serve as both an intuitive and quantitative aid to interpreting results from the complex model. For example, the contrast between models highlights the role of technically complex assumptions. To some extent, errors in the age structure data cancel in calculating the mean age. Thus, our analysis suggests options available for investigating stocks where only summary age data are reliable.

Résumé : L'analyse de l'âge à la capture est devenue un outil courant pour évaluer les stocks de poissons d'importance commerciale. Mais les modèles modernes de l'âge à la capture peuvent contenir des dizaines, sinon des centaines, de paramètres à évaluer. On compare dans la présente étude la performance d'un modèle complexe typique et d'un modèle plus simple, où la structure d'âge de la population n'est représentée que par l'âge moyen annuel. Il apparaît que la trajectoire de la population estimée d'après le modèle simple concorde généralement avec la trajectoire obtenue par l'analyse de l'âge à la capture. Cela indique que le modèle simple peut servir d'aide quantitative et intuitive pour interpréter les résultats du modèle complexe. Par exemple, le contraste entre les modèles met en luminère le rôle des hypothèses techniquement complexes. Dans une certaine mesure, des erreurs dans les données sur la structure d'âge s'annulent dans le calcul de l'âge moyen. Notre analyse fait ressortir des options disponibles pour étudier des stocks lorsque seules les données sommaires sur l'âge sont fiables.
[Traduit par la Rédaction]

Introduction

Catch-age analysis has become a standard tool of modern fisheries stock assessment (Megrey 1989). The analysis attempts to reconstruct population numbers at age and time, based on fishery catch, sampled age proportions, and other ancillary data. Implementations range from deterministic back-calculations, such as sequential population analysis (Gulland 1965) and cohort analysis (Pope 1972), to the statistical models of Fournier and Archibald (1982), Deriso et al. (1985), and Methot (1989, 1990). More recent approaches consider both process and measurement errors (Pella 1993; Schnute 1994), either in a likelihood context (Sullivan 1992; Gudmundsson 1994; Schnute and Richards 1995) or in a Bayes context (McAllister et al. 1994; McAllister and Ianelli 1997; Punt and Hilborn 1997).

This paper articulates theoretical and practical differences between historical and modern analyses. In particular, we compare the performance of a typically complex catch-age model with a simpler model described by Schnute and Richards (1998) in which the population age structure is represented only by the mean annual age. Our simple model is based on moment equations similar to those found by Deriso (1980), Schnute (1985, 1987), and Fournier and Doonan (1987), as reviewed by Hilborn and Walters (1992, chap. 9). In particular, we assume knife-edged recruitment to the fished population whereas catch-age analyses typically assume that recruitment occurs over a range of ages. Using data from a real population, we find that reconstructed population trajectories are remarkably similar for the simple and complex models. However, recruitment trends estimated by the simple model are somewhat smoother than those inferred from the complex model.

Simple models can serve as intuitive and quantitative aids to interpreting results from complex models. For example, analytical results from a simple model can guide the understanding of a complex model, where analytical results are not available. In addition, differences in numerical results highlight the role of technically complex assumptions.

Where possible, we choose previously published models and data to simplify our presentation. Our data set and complex model are described in Richards et al. (1997), where the model has been adapted from Schnute (1994) and Schnute and Richards (1995) to address specific features of the data. The data here, updated from Richards and Olsen (1996) and Richards et al. (1997), are selected from the fishery for Pacific ocean perch (*Sebastes alutus*) in southern Queen Charlotte Sound, British Columbia, Canada. Our simple model derives from moment equations discussed by Schnute and Richards (1998).

Section 1 describes the Pacific ocean perch data set and data preprocessing requirements. Our simple model is defined and tailored for the Pacific ocean perch data in Section 2, based on mathematical derivations in the Appendix. Results of the model comparison are presented in Section 3. We discuss further implications of our analysis in Section 4.

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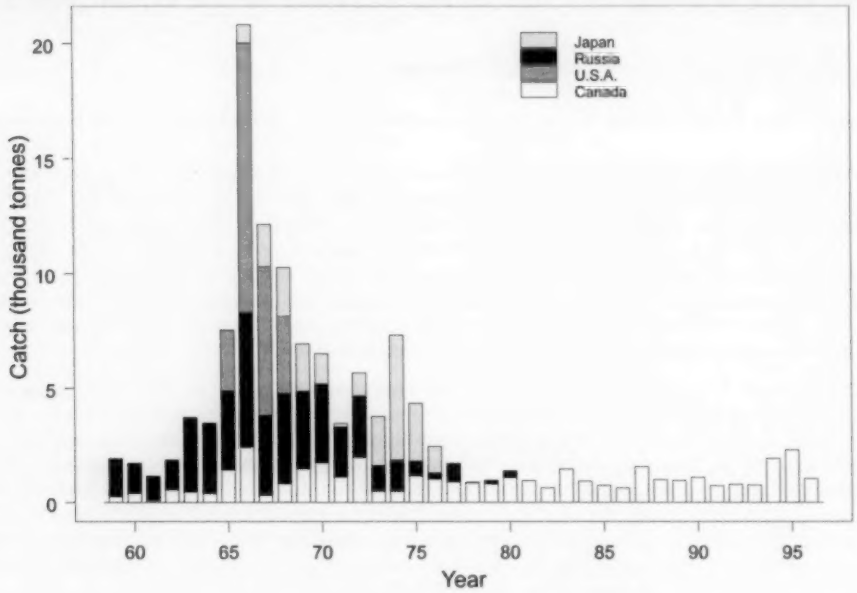
Fig. 1. Pacific ocean perch trawl catch reported by nation from southern Queen Charlotte Sound, British Columbia, between 1959 and 1996.



1. Data

Fishery catch and proportions at age sampled from the catch constitute the core data for any catch-age analysis. We have updated these Pacific ocean perch data from Richards et al. (1997) to include information from the most recent year (1996). The catch data in Fig. 1 show that the stock was subjected to a large multinational fishery until the mid-1970s, followed by a comparatively small Canadian fishery. Large catches were harvested by Soviet and Japanese fleets during the late 1960s and early 1970s, prior to extended jurisdiction. Other rockfish (*Sebastes* spp.) stocks along the Pacific coast of North America were also targeted by Soviet and Japanese fleets at this time. Several studies in the early 1980s (Ketchen 1981; Kimura 1981; Archibald et al. 1983) indicated depletion of the Pacific ocean perch stock by the late 1970s. The subsequent Canadian fishery was therefore restricted by low annual quotas and other management measures.

Two different methods have been used to determine the Pacific ocean perch age proportions illustrated in Fig. 2. Between 1963 and 1976, ages were obtained from surface readings of otoliths (Archibald et al. 1983), a method known to underestimate true ages older than about 15–16 years (Stanley 1987). Between 1977 and 1996, ages were obtained by the break and burn method (Chilton and Beamish 1982). Actual break and burn ages range up to 79 years. However, ages 29 years and older are grouped in Fig. 2, consistent with the maximum observed surface ages. The two blank columns portray the absence of age data for 1986 and 1988.

In Fig. 2, the modal age-class within a year is always older than the age-class at first recruitment, indicating that availability to the fishery increases with age for younger ages. Our complex model for these data, as in most catch-age analyses, uses the complete age proportion data set and allows selectivity by the fishery to vary with age. However, our simple model uses only the mean age and requires the assumption of knife-edged recruitment. We selected age 9 years as the age at recruitment for the simple model because strong year-classes often become apparent at age 9 years, particularly in 1970 and 1985.

We make two simplifications in the calculation of mean age, portrayed as the solid line in Fig. 2. First, to accommodate some of the uncertainty in true ages from surface readings, we assign an age of 29 years to all fish in the accumulator age-class. Second, we assign an age of 9 years to all fish younger than the age at recruitment. In this scenario, we assume that the small proportions at age 7–8 years actually represent fish of age 9 years that were incorrectly aged. Figure 2 provides some supporting evidence; the largest proportions at age 8 years occur in 1970, 1985, and 1989, years for which a large year-class has recruited at age 9 years. Such a pattern is suggestive of ageing error rather than consecutive strong year-classes. An alternative approach might be to exclude data on fish younger than age 9 years from both the total catch and the age proportion data. These procedures lead to similar results and we only report the ageing error scenario in the discussion below.

The trend in mean age in Fig. 2 contains interesting differences from the year-class trends. Mean age is essentially constant for the 1963–1976 period; the strong year-class appearing in 1970 acts to counterbalance the increasing age of earlier strong year-classes. Mean age increases between 1976 and 1983, and the subsequent decrease in mean age until 1987 coincides with the appearance of the next strong year-class. Obviously, the relatively large proportions in the accumulator age-class influence the mean age calculation.

Catch and age proportion data alone are generally inadequate to reconstruct the history of a fish population. Two series of research trawl surveys are also available to index Pacific ocean perch abundance (Fig. 3). The first series was conducted

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Fig. 2. Pacific ocean perch age proportions sampled from the Canadian catch for recruited ages of 7 years and older and the mean age (solid line), computed as discussed in the text. The radius of a circle is proportional to the measured age proportion, where data are scaled to sum to 1 across each column and ages 29 years and older are grouped. The vertical dotted line distinguishes age data obtained by surface readings of otoliths (1963–1976) from later break and burn readings (1977–1995). No age proportion data are available for 1986 or 1988.

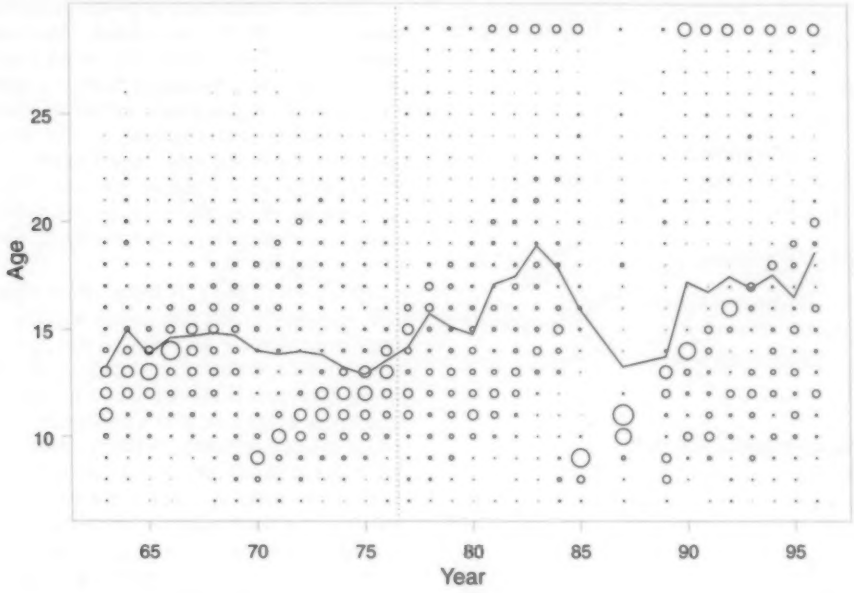
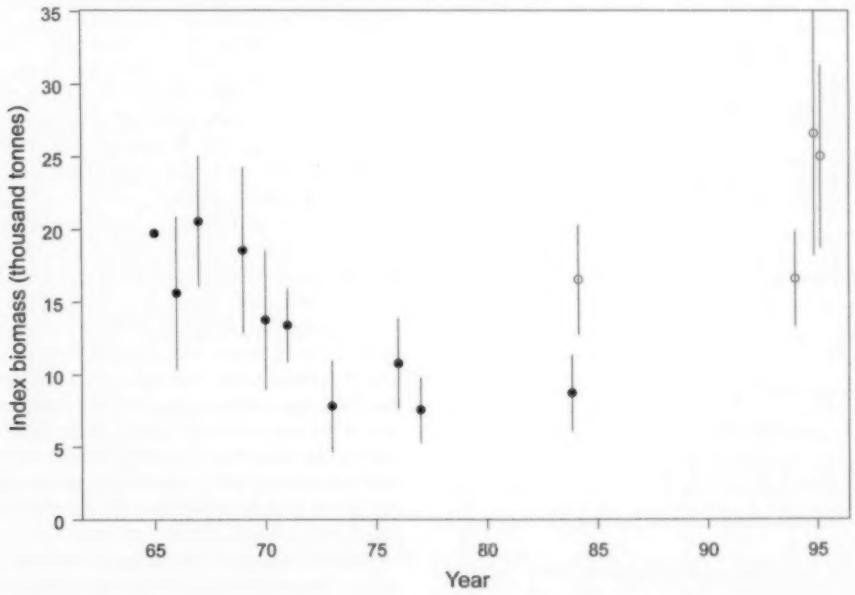


Fig. 3. Point estimates and 95% confidence intervals for the Pacific ocean perch trawl abundance indices obtained by swept-area trawl surveys using a research vessel (solid circles) and chartered commercial vessels (open circles).



by a research vessel between 1965 and 1984. The second series was conducted by a chartered commercial vessel, with a calibration survey in 1984 and subsequent surveys in 1994 and 1995 (Hand et al. 1995; Yamanaka et al. 1996). Confidence intervals in Fig. 3 are determined from standard application of sampling theory for a stratified random design (e.g., Cochran 1977, chap. 5). The confidence interval is missing for the first survey because only one sample tow was completed in one of the four survey depth strata for that year. At best, these intervals provide a minimal estimate of the true survey variance;

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Table 1. Mean age model, assuming knife-edged recruitment.

Data

(T.1) $x_t = \sum_a a p_{at}$

(T.2) $w_t = \sum_a w_{at} p_{at}$

(T.3) D_t; $C_t = D_t / w_t$

(T.4) (I_{it}, f_{it}); $t \in \mathbf{T}_i$; $i = 1, 2$

Parameters

(T.5) $\boldsymbol{\Theta} = (M, q_1, q_2, A_1, P_1, \{R_t\}_{t=1}^{T}, R, \gamma, \rho, \kappa, \tau_2)$

(T.6) $\sigma^2 = \rho\kappa^2$, $\tau_1^2 = (1 - \rho)\kappa^2$

(T.7) $\kappa^2 = \sigma^2 + \tau_1^2$, $\rho = \dfrac{\sigma^2}{\sigma^2 + \tau_1^2}$

Unobserved states

(T.8) R_t, S_t, P_t, B_t, A_t

Initial states ($t = 1$)

(T.9) $B_1 = w_1 P_1$

(T.10) $S_1 = P_1 - C_1$

State dynamics ($2 \le t \le T$)

(T.11) $R_t = R^{1-\gamma} R_{t-1}^{\gamma} e^{\sigma\delta_t}$

(T.12) $P_t = R_t + e^{-M} S_{t-1}$

(T.13) $B_t = w_t P_t$

(T.14) $A_t = 1 + \dfrac{P_t - R_t}{P_t} A_{t-1}$

(T.15) $S_t = P_t - C_t$

Observations

(T.16) $I_{it} = [q_i (B_t - f_{it} D_t)] e^{\tau_1 \varepsilon_{it}}$; $t \in \mathbf{T}_i$; $i = 1, 2$

(T.17) $x_t = A_t e^{\tau_2 \eta_t}$; $t \ge 1$

Likelihoods

(T.18) $L_1(\mathbf{Q}) = \prod_{t=2}^{T} \left[\dfrac{1}{(2\pi)^{1/2}\sigma} \exp\left(-\dfrac{1}{2}\delta_t^2 \right) \right]$

(T.19) $L_2(\mathbf{Q}) = \prod_{i=1}^{2} \prod_{t \in \mathbf{T}_i} \left[\dfrac{1}{(2\pi)^{1/2}\tau_1} \exp\left(-\dfrac{1}{2}\varepsilon_{it}^2 \right) \right]$

(T.20) $L_3(\mathbf{Q}) = \prod_{t=1}^{T} \left[\dfrac{1}{(2\pi)^{1/2}\tau_2} \exp\left(-\dfrac{1}{2}\eta_t^2 \right) \right]$

(T.21) $L(\mathbf{Q}) = \prod_{i=1}^{3} L_i(\boldsymbol{\Theta})$

(T.22) $\ell(\mathbf{Q}) = -2 \log L(\boldsymbol{\Theta}) - K$

$= (T - 1)\log(\sigma^2) + (T_1 + T_2)\log(\tau_1^2) + T \log(\tau_2^2)$

$+ \sum_{t=2}^{T} \delta_t^2 + \sum_{i=1}^{2} \sum_{t \in \mathbf{T}_i} \varepsilon_{it}^2 + \sum_{t=1}^{T} \eta_t^2$

they incorporate only the variation in catch rates among survey tows within a given year.

2. Model development

The simple model is presented in Table 1, where the prefix "T" is used to distinguish tabulated equations from those in the text. Table 1 is divided into seven sections that describe the input data, model parameters, unobserved states, initialization assumptions, state dynamics, observation equations, and corresponding likelihood functions. This framework follows the state space design principles described by Schnute (1994).

The data section must be tailored to represent each specific data set. For Pacific ocean perch, input data include the age proportions p_{at} and weights w_{at} for each age-class a and year t, the annual catch biomass D_t, the two abundance index survey series (I_{1t}, I_{2t}) available for sets of years ($\mathbf{T}_1$, $\mathbf{T}_2$), and the corresponding catch fractions (f_{1t}, f_{2t}) harvested prior to the survey during survey years. Age-classes $a = 1,...,21$ correspond to ages 9–29 years, where the raw data have been preprocessed as described in Section 1 and the last age-class acts as an accumulator. Years $t = 1,...,T$ correspond to the 1963–1996 period, so that $T = 34$.

Equations (T.1)–(T.2) define observations of the mean annual age-class x_t and fish weight w_t, respectively, where the age proportions are defined such that $\Sigma_a p_{at} = 1$ for each year t. Values of x_t differ by a constant 8 units from the mean age in Fig. 2 because of the conversion between age and age-class. The mean weights w_t play a minor role in the simple model, which is based primarily on fish numbers. However, values w_t are required to convert catch biomass D_t to catch numbers C_t in (T.3). Because age data are missing for 1986 and 1988, values w_t for those years are interpolated from adjacent years.

The state dynamics for the simple model are drawn primarily from Schnute and Richards (1998), who present deterministic models only. The stochastic assumptions in Table 1 closely parallel those in the complex model (Schnute and Richards 1995; Richards et al. 1997). We assume that process error σ in the recruitment complements measurement error τ_1 in the survey abundance index. Intuitively, apparent variations in abundance can be explained primarily though variability in recruitment (high process error σ) or indexing (high measurement error τ_1). Such ambiguities are inherent to most models that include both measurement and process error (Kendall and Stuart 1979, chap. 29). We resolve this ambiguity by fixing the variance ratio ρ between recruitment and total variance κ^2 through the relationships (T.6)–(T.7). A value of ρ near 1 corresponds to high variance in recruitment and low variance in the model fit to the survey index. Conversely, a low value of ρ corresponds to near constant recruitment and high variance in the survey index fit.

Our simple model includes five unobserved state variables (T.8) for each year t. Recruitment R_t occurs at the start of the year, resulting in the population P_t, biomass B_t, and mean age A_t. After the catch is removed, the survivors S_t remain at the end of the year and experience natural mortality M prior to the start of the following year. The model parameter vector $\boldsymbol{\Theta}$ in (T.5) includes all recruitments R_t, plus the initial states P_1 and A_1. These give initial values for the remaining two states B_1 and S_1 in (T.9)–(T.10).

Because the unobserved state variables R_t are also treated as explicit parameters, they do not require an update equation. The model equation (T.11) serves only to identify the recruitment variance σ^2, rather than to describe a specific biological process. To allow possible recruitment correlation, we consider the lognormal autoregressive process

$$\log R_t = \log R + \gamma(\log R_{t-1} - \log R) + \sigma\delta_t$$

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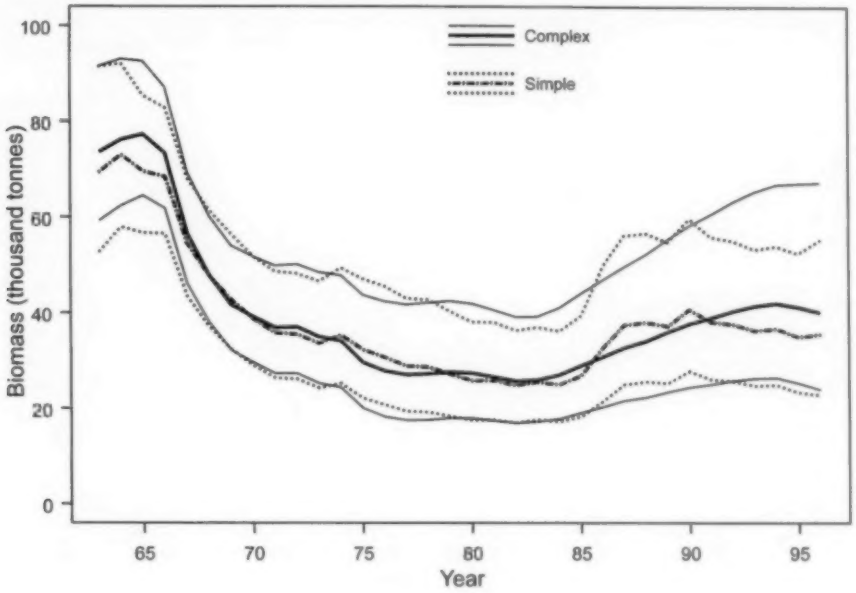
Fig. 4. Estimates and 95% confidence intervals from the simple and complex models for Pacific ocean perch exploitable biomass between 1963 and 1996.



equivalent to (T.11), where the standard normal variates δ_t are assumed independent.

Equations (T.12)–(T.15) sequentially update the remaining four state variables (P_t, B_t, A_t, S_t) for $t = 2,...,T$. Thus, the population P_t in (T.12) at the beginning of year t consists of the recruits R_t and the survivors $e^{-M}S_{t-1}$ from the previous year. Equation (T.13) converts P_t to biomass B_t for comparison with the indices I_{it}, where we use the sample weights w_t to represent the population mean weight in year t. The update equation (T.14) for mean age A_t is derived in the Appendix, which also shows that (T.14) is consistent with (T.12). Intuitively, (T.14) states that A_t is computed by adding 1 year to an adjusted value of the previous mean age A_{t-1}. The adjustment depends on the proportion of recruits R_t in the total population P_t. For example, if $R_t = 0$, then $A_t = 1 + A_{t-1}$ because the population includes only survivors that have gained 1 year in age. Similarly, if $P_t = R_t$, then $A_t = 1$ because the population consists entirely of new recruits. Thus, A_t is subject to the constraint

$$(2.1)\quad 1 \le A_t \le A_{t-1} + 1$$

The final update equation (T.15) simply defines the survivors remaining after the fishery.

The observation equations (T.16)–(T.17) relate the population states to the measured observations. We assume that the trawl index surveys I_{it} ($i = 1, 2$) are proportional to the population biomass after a fraction f_{it} of the catch has been removed. The parameters q_i calibrate the surveys to biomass units. The lognormal standard error τ_1 is assumed to apply to both surveys with independent standard normal deviates ε_{it}. Similarly, we assign error to the observed age x_t in (T.17) through a lognormal measurement with standard error τ_2 and standard normal deviates η_t.

Our model distinguishes the observed mean age x_t from the population mean A_t. By contrast, we make no distinction between the observed mean weight w_t and the population mean weight, which we denote by W_t. In effect, we assume that $W_t = w_t$. We could have defined dynamic and observation equations for W_t and w_t, respectively, analogous to (T.14) and (T.17) for A_t and x_t. For example, Schnute and Richards (1998) provide an update equation for population weight moments assuming von Bertalanffy growth. Unlike (T.14), the update equation for W_t requires additional parametric assumptions about the form of the age–weight relationship. We are reluctant to impose such assumptions for Pacific ocean perch. Indeed, we obtained the observations w_{at} from a nonparametric smoother through the raw sample data. Furthermore, given the longevity of Pacific ocean perch and their strongly asymptotic growth, more contrast is possible in mean age A_t than in mean weight W_t.

Equations (T.18)–(T.21) define the likelihood function $L(\mathbf{Q})$ for this stochastic model from the parameter vector $\mathbf{Q}$ in (T.5). Calculation of $L(\boldsymbol{\Theta})$ involves solving (T.11), (T.16), and (T.17) for the standard normal deviates δ_t, ε_{it}, and η_t. Equations (T.6)–(T.7) allow $\boldsymbol{\Theta}$ to be expressed in terms of either (σ, τ_1) or (κ, ρ). In the analyses here, we fix ρ (Richards et al. 1997) and allow κ to vary. For estimation, we use the function $\ell(\boldsymbol{\Theta})$ in (T.22), which is equivalent to twice the negative log likelihood except for a constant K. The quantity $T_1 + T_2$ in (T.22) denotes the total number of surveys in both survey series.

Table 1 omits one technical detail for the Pacific ocean perch case. Two years of age data are missing. We omit these years in the calculations (T.17). Thus, we reduce the likelihood (T.20) by two terms and make corresponding changes in (T.22).

Our complex catch-age analysis of Pacific ocean perch is completely described in Richards et al. (1997), based on the model of Schnute and Richards (1995). The state dynamic

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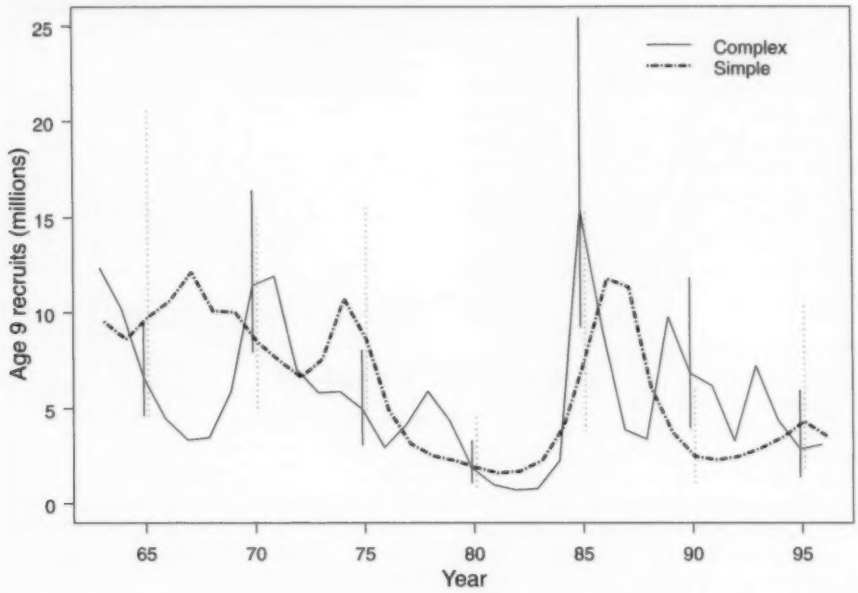
Fig. 5. Estimates of age 9 recruitment from the simple and complex models, with corresponding 95% confidence intervals (vertical lines). To simplify the display, confidence intervals are indicated for every fifth year only.



equations are expressed in terms of numbers N_{at} at both age a and time t. Recruitment is not knife-edged; rather, selectivity by the fishery increases asymptotically with age in a given year. Furthermore, the initial states must allow calculation of N_{a1} for each a; the model accomplishes this through estimation of historic recruitments R_{2-a}. The observation equation (T.17) is replaced by a comparison between the predicted proportions at age determined from the state dynamics and the observations portrayed in Fig. 2. We use a multivariate logistic function for this comparison.

For Pacific ocean perch with 34 years of data, the parameter vector $\boldsymbol{\Theta}$ in (T.5) for the simple model contains 44 terms, while the comparable parameter vector for the complex model contains 54 terms. For both models, we fix the parameter $\rho = 0.9$, following the rationale in Richards et al. (1997). All other terms in $\boldsymbol{\Theta}$ are treated as parameters to be estimated. In particular, we estimate the natural mortality rate M. Computationally, we require procedures to estimate $\boldsymbol{\Theta}$ by minimizing $\ell(\boldsymbol{\Theta})$ in (T.22) and the comparable negative log likelihood for the complex model. We obtain these estimates with the software package AD Model Builder (Otter Research Ltd. 1994), which provides automatic derivatives (Griewank and Corliss 1991) for C++ code and a gradient-based search algorithm. The software also facilitates the computation of confidence intervals for parameter estimates and derived quantities based on normal approximations (Richards et al. 1997, appendix B).

3. Results

Results in Fig. 4 for the complex model indicate that Pacific ocean perch biomass declined during the late 1960s and early 1970s from historical highs in the 1960s. Biomass was relatively stable during the late 1970s and early 1980s and then increased through the early 1990s with a slight decline in 1996. However, uncertainty increases for the more recent estimates; 95% confidence intervals are broader for estimates for the late 1980s and 1990s. The biomass reconstruction for the simple model is similar. Initial biomass estimates are slightly lower, but the two models produce essentially identical estimates and confidence intervals for the period between the late 1960s and early 1980s. Biomass estimates in the final year are slightly lower for the simple model and the confidence interval is narrower. The two models closely agree on the lower confidence interval for the 1996 biomass, but the simple model appears more certain that the biomass has not increased to high levels.

One reason that biomass estimates are similar for the two models is that estimates of M are also similar. Estimates and standard errors are 0.073 ± 0.016 and 0.070 ± 0.016 for the simple and complex models, respectively. In these types of models, M acts as a scale factor; a higher value of M implies that a higher biomass is required to sustain a given catch.

A feature that distinguishes the trajectories of the two models in Fig. 4 is the smoothness of the trend. For example, the simple model suggests that biomass increased sharply in the period 1985–1987 and then remained relatively stable. By contrast, the selectivity function for the complex model allows recruits of a given year-class to enter the exploitable population over a multiyear period. Essentially, the selectivity function acts to smooth changes in abundance due to recruitment.

Estimated trends in age 9 recruitment are somewhat different for the two models, although the average recruitment is similar (Fig. 5). For the complex model, peak age 9 recruitments are associated with years 1970–1971, 1985, 1989, and 1993. These correspond to relatively large proportions of age 9 fish in the input data (Fig. 2). Recruitment trends are to some extent related to ageing error. For example, the complex model cannot distinguish a strong year-class with ages known to ± 1 year from two or three consecutive above-average year-classes. For

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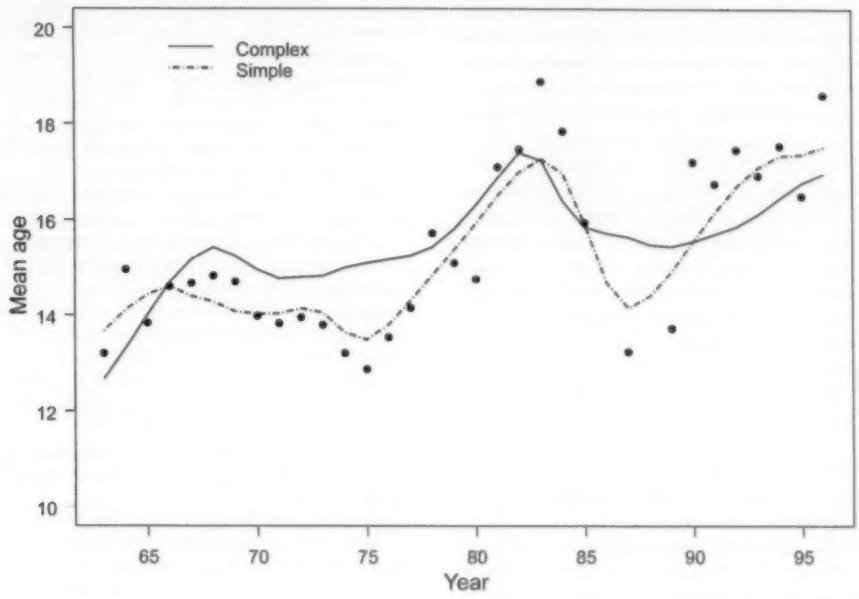
Fig. 6. Estimates of mean age from the simple and complex models and the observed mean age (circles).



the simple model, peak recruitments lag those of the complex model. However, recruitment estimates are more uncertain for the simple model, as indicated by the broader 95% confidence intervals.

Richards et al. (1997) present a residual analysis for the complex model. For the simple model, the key residuals are those associated with the prediction of mean age. The predicted mean age appears as a smooth function of the observed mean age (Fig. 6). Given the upper limit in the constraint (2.1), the predicted mean age cannot match the large observed changes in mean age in the 1980s; the simple model associates these discrepancies with observation error. Predicted mean ages for the complex model fit the observed data poorly. The complex model overestimates mean age for most years with otolith surface age determination and underestimates mean age for most years with break and burn age determination. This pattern is an artifact of different choices of accumulator age-classes for surface and break and burn data (age 16 and 29 years, respectively). Similarly, the increasing trend in predicted mean age at the beginning of the series for the complex model can be attributed to the model's internal accounting for the transition between the two accumulator age-classes.

4. Discussion

We have distinguished simple from complex models based on the input data and the number of states required to account for the underlying dynamics. For example, a typical catch-age model involves observed age proportions p_{at} for A age-classes and T years, giving a total of $(A-1)T$ independent data values. In the simple model here, this large data matrix reduces to the vector of T observed mean ages x_t. Similarly, a catch-age model typically involves AT states N_{at} that represent the population at age a in year t. This dimensionality is reduced to $2T$ in our simple model by considering recruits R_t and survivors S_t only. Simplified model equations accompany these reductions in dimensionality. For example, fishery selectivity is not considered in our simple model.

Model simplicity can also pertain to the number of explicit parameters. For example, our simple model includes the T recruitments R_t in the parameter vector $\boldsymbol{\Theta}$. This number could be reduced considerably by assuming constant recruitment; thus, (T.11) could be replaced by

$$(4.1)\quad R_t = R.$$

Such a simplified model for the Pacific ocean perch data leads to unrealistically high biomass estimates. For example, under the assumption (4.1), the biomass estimate at the beginning of 1996 is 113 200 tonnes, substantially higher than the upper 95% confidence interval for the corresponding simple or complex model estimates (Fig. 4). Without variable recruitment to explain historical high catches, the model produces consistently high biomass estimates to accommodate the entire catch history. Thus, although assumption (4.1) reduces the number of parameters, it is too simplistic for the data analyzed here.

Our analysis has shown that a model based on mean annual age can reconstruct biomass trends similar to reconstructions from a full age-structured model. While the analyst might rightly prefer a realistic complex model for fisheries stock assessment, a simple model can still provide a useful tool. In particular, it can illustrate the role of specific assumptions in a complex model. For example, the addition of a selectivity function to our complex model does not appear to alter the model biomass estimates. However, the selectivity function and full age structure do contribute to more realistic estimation of annual recruitments.

Ageing error is apparent in the Pacific ocean perch data set (Fig. 2), where it acts to smooth the age proportions. Thus, the abundance of a peak year-class is typically underestimated and adjacent year-class abundances are overestimated (Richards et

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al. 1992). Our complex model does not contain specific adjustments for ageing error. Consequently, it tends to spread the influence of a strong year-class over adjacent year-classes (Fig. 5). To some extent, ageing errors and other sampling errors in the age structure cancel in the mean age calculation (T.1). Thus, our simple model suggests options for investigating stocks where only summary age data are reliable.

Age data, even in summary form, are not available for many fish stocks, whereas weight data are almost always available. Thus, following Schnute and Richards (1998), our update equation for mean age could potentially be replaced or supplemented by analogous equations for mean weight or length. Such update equations could also be extended to acknowledge environmental influences on the growth process. Similarly, observation equations for mean weight or length could be tailored to accommodate sample characteristics. Fish age, when available, generally provides more information about underlying population dynamics than fish size. For example, age of an individual fish increases linearly each year, but its weight typically becomes asymptotic at older ages. For Pacific ocean perch, mean age is a much more informative population characteristic than mean weight.

Preprocessing of the input data is required for any analysis. For the simple model, these requirements are more stringent than for the complex model. For example, the choice of age at recruitment for the simple model may not be obvious. The fishery catch of any fish younger than the age at first recruitment must also be acknowledged. We considered various preprocessing methods for the Pacific ocean perch data and obtained essentially similar biomass estimates. We opted to attribute ageing error to the catch of fish younger than the age at recruitment. In other situations, the prerecruit catch could be removed from the fishery catch, with the age proportions adjusted to sum to 1 over the recruited ages.

It could be argued that our complex model is too simple, with only 10 parameters more than the simple model. Certainly, we could extend our complex analysis to include ageing error, a selectivity function with more than two parameters, or even survival process error as suggested by Schnute and Richards (1995). Of course, there is a trade-off between increased realism and ease of interpretation. In this paper, we have suggested ways of collapsing a complex analysis such that the consequences of added features can be readily understood. We envision a progressive development of complex models, in which the relevance of added complexity is investigated at each stage.

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Appendix. Dynamic equations

In an age-structured population, let N_{at} denote the number of fish in age-class a at the start of year t. Recruits R_t, total population P_t, mean age A_t, and survivors S_t are then given, respectively, by

(A.1) $R_t = N_{1t}$,

(A.2) $P_t = \sum_{a=1}^{\infty} N_{at}$,

(A.3) $A_t = \frac{1}{P_t} \sum_{a=1}^{\infty} aN_{at}$,

(A.4) $S_t = P_t - C_t$,

where age-class $a = 1$ corresponds to the recruitment age and C_t is the annual catch. If fishing is not selective on age-classes, it follows that for $a \geq 1$:

(A.5) $N_{a+1,t+1} = e^{-M} \frac{S_t}{P_t} N_{at}$.

From (A.1)–(A.2) and (A.5), it follows that

$$P_t = N_{1t} + \sum_{a=1}^{\infty} N_{a+1,t}$$

$$= R_t + e^{-M} \frac{S_{t-1}}{P_{t-1}} \sum_{a=1}^{\infty} N_{a,t-1}$$

$$= R_t + e^{-M} S_{t-1}.$$

This proves (T.12). Similarly, starting from (A.3) and (A.5), the calculation

$$A_t = \frac{1}{P_t}\left[R_t + \sum_{a=1}^{\infty} (a+1)N_{a+1,t}\right]$$

$$= \frac{1}{P_t}\left[R_t + e^{-M} \frac{S_{t-1}}{P_{t-1}} \sum_{a=1}^{\infty} (a+1)N_{a,t-1}\right]$$

$$= \frac{1}{P_t}\left[R_t + e^{-M} \frac{S_{t-1}}{P_{t-1}} (P_{t-1}A_{t-1} + P_{t-1})\right]$$

(A.6) $$= \frac{1}{P_t}[R_t + (P_t - R_t)(A_{t-1} + 1)]$$

$$= 1 + \frac{P_t - R_t}{P_t} A_{t-1}$$

proves (T.14), where step (A.6) uses (T.12).

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Phytoplankton responses to nutrient and grazer manipulations among northeastern lakes of varying pH

Paul Bukaveckas and William Shaw

Abstract: Short-term nutrient enrichment and zooplankton exclosure experiments were conducted at 14 lakes representing various stages of acidification (pH 4.6–6.8). We measured changes in chlorophyll as an indicator of the severity of nutrient limitation and grazing intensity and compared these with independent measures of P limitation (cell P quotas and phosphatase activity) and grazing (zooplankton densities and inferred community grazing rates). Results from nutrient enrichment experiments showed good correspondence to measured phosphatase activity but not cell P quotas. Phytoplankton in acidic lakes (pH < 5.0) responded more strongly to nutrient enrichment and exhibited fourfold higher biomass-specific phosphatase activity compared with nonacidic lakes. Phytoplankton responses to the removal of macrozooplankton did not exhibit any consistent pattern among lakes of varying acidity. Although the largest herbivores (*Daphnia*) were more abundant in nonacidic lakes, their absence in acidic lakes was compensated for by increased abundance of smaller species (principally *Diaptomus minutus* and *Bosmina longirostris*). The magnitude of the response to grazer removal was positively correlated with lake zooplankton densities at the time of the experiment, and the use of a nonlinear model enabled us to explain 84% of the variation in this response among the 14 sites.

Résumé : Nous avons fait de courtes expériences d'enrichissement et d'exclusion du zooplancton dans 14 lacs acidifiés à divers degrés (pH allant de 4,6 à 6,8). Nous avons mesuré les changements de la teneur en chlorophylle, paramètre considéré comme un indicateur de la gravité de la limitation en nutriants et de l'intensité du broutage, et nous avons comparé les fluctuations observées à des mesures de la limitation en P (P cellulaire et activité phosphatase) et du broutage (densité du zooplancton et, par déduction, taux de broutage attribuable à cette communauté) faites indépendamment. Les expériences d'enrichissement ont permis de mettre en évidence une bonne correspondance avec l'activité phosphatase, mais non avec la concentration de P cellulaire. Dans les lacs acides (pH < 5,0), le phytoplancton a réagi plus fortement à l'enrichissement, et l'activité phosphatase de la biomasse était quatre fois plus élevée que dans les lacs non acides. Dans les lacs acidifiés à divers degrés, nous avons constaté que la réaction du phytoplancton à l'élimination du macrozooplancton ne présentait pas de caractéristiques constantes. Les plus gros herbivores (*Daphnia*) étaient surtout abondants dans les lacs non acides, et, dans les lacs acidifiés, leur absence était compensée par l'abondance accrue des espèces de moins grande taille (essentiellement *Diaptomus minutus* et *Bosmina longirostris*). Nous avons constaté une corrélation positive entre l'importance de la réaction déterminée par l'élimination des brouteurs et la densité des populations zooplanctoniques au moment de l'expérience, et, avec un modèle non linéaire, nous avons pu expliquer 84 % de la variation mise en évidence dans les réactions observées aux 14 stations.
[Traduit par la Rédaction]

Introduction

Experimental manipulations of nutrients and zooplankton have been used to investigate factors regulating lake phytoplankton dynamics for over two decades (e.g., Schelske and Stoermer 1972). Experimental approaches can be a powerful tool in field studies because experiments can be repeated in time and space to delineate patterns along spatial and temporal gradients. A number of investigators have used this approach to investigate seasonal variation in phytoplankton responses to nutrient additions and manipulations of zooplankton density (e.g., Sarnelle 1993). A few studies have used spatially replicated experiments to investigate phytoplankton responses in large lakes (e.g., Aldridge et al. 1995). Small-scale experiments have also been incorporated within broader designs such as whole-lake manipulations to investigate specific mechanisms regulating population- and community-level responses (Sierszen and Frost 1990). Experimental approaches have largely been neglected in comparative lake surveys. Therefore, attempts to identify generalizable patterns over broad environmental gradients have relied on literature compilations in which experiments differing in the specifics of their design (size of enclosure, number of replicates, etc.) were analyzed to identify emergent properties (Elser et al. 1990; Cyr and Pace 1992).

In this paper, we present data from experiments that were replicated along a spatial gradient represented by 14 lakes of varying acidity. Our knowledge of the utility of small-scale manipulative experiments in the context of acidification assessment is very limited beyond the laboratory bioassays that

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Table 1. Site characteristics of 14 lakes selected for field experiments.

Site	Surface area (ha)	Max. depth (m)	pH	ANC (μequiv.·L^{-1})	Total Al (μg·L^{-1})	Monomeric Al (μg·L^{-1})	kd (m^{-1})	TDP (μg·L^{-1})	DIN (μg·L^{-1})	Chl (μg·L^{-1})	No. of experiments (total $N = 40$)
Cone Pond (CO)	3	7	4.6	−25	500	400	0.25	3	50	0.92	3
Cranberry Pond (CR)	7	9	4.8	−15	250	70	0.27	7	332	0.37	4
Silver Lake (SL)	21	15	4.8	−15	320	280	0.21	3	579	0.26	2
Dart's Lake (DR)	52	15	5.1	−10	170	90	0.50	6	491	0.90	7
Thirsty Pond (TH)	11	3	5.3	0	120	50	0.42	10	116	1.18	2
Twitchell Lake (TW)	58	11	5.4	0	120	50	0.62	9	273	0.97	2
Limekiln Lake (LI)	187	20	5.6	0	—	—	0.24	—	—	0.69	1
Duck Lake (DU)	137	3	6.0	+30	60	10	0.74	10	33	6.33	2
Arbutus Lake (AR)	50	8	6.2	+70	20	10	0.69	7	170	2.96	1
McBride Pond (MB)	2	3	6.3	+40	50	10	0.47	13	60	1.88	1
Moss Lake (MO)	46	15	6.5	+70	50	10	0.54	12	422	1.76	3
Woods Lake (WD)	23	11	6.5	+180	60	15	0.52	9	363	1.27	8
Wolf Lake (WF)	57	7	6.6	+80	20	10	0.36	5	378	1.72	1
Mirror Lake (MI)	15	11	6.8	+70	20	10	0.55	8	80	2.35	3

Note: kd, light attenuation coefficient; DIN = N-NO_3 + N-NH_4.

test for toxic effects on target organisms (but see Gonzalez and Frost 1994). Recent findings suggest that current mandates for reducing emissions may be inadequate to promote recovery or prevent further acidification due to long-term depletion of base cations from soils (Likens et al. 1996). Hence, there is continued interest in the effects of acidification on ecosystem structure and functioning.

Effects of acidification on plankton community structure have been well documented and there is broad consensus that increases in lake acidity are accompanied by reductions in species richness (Havens 1992; Locke 1992). The patterns of species loss/replacement result in zooplankton communities in acidic lakes that lack large-bodied herbivores (e.g., daphnids) and are dominated by relatively small species (Keller et al. 1990; Pinel-Alloul et al. 1990). The loss of acid-sensitive phytoplankton species results in communities dominated by large-celled individuals and in some cases by small (nonfilamentous) blue-green algae (Blomqvist et al. 1995). The pronounced shifts in community structure have led some to argue that community- and ecosystem-level functioning (primary production, zooplankton grazing, trophic transfer efficiencies) may be impaired in chronically acidified lakes (Havens 1992; Blomqvist 1996). It has also been suggested that changes in lake chemistry, particularly elevated concentrations of monomeric Al, may impair P cycling, either directly through complexation reactions or by inhibiting alkaline phosphatase activity, and thereby diminish primary production (Broberg and Persson 1984; Jansson et al. 1986). Results from whole-lake experiments have generally contradicted these arguments, showing little change in community functioning despite changes in dominant species (Schindler et al. 1991). Functional redundancy within grazer and primary producer groups may act to maintain community-level characteristics (biomass, production, grazing) despite substantial reductions in species richness (Frost et al. 1995).

We conducted field experiments to test three hypotheses: (*i*) that phytoplankton responses to nutrient enrichment and zooplankton exclosure would exhibit greater variation among sites than is observed seasonally at a single site, (*ii*) that elevated Al concentrations in acidified lakes would result in more severe P limitation as indicated by stronger responses to P enrichment, and (*iii*) that the absence of large, filter-feeding herbivores from acidified lakes would result in reduced grazer control as indicated by weak responses to zooplankton removal. We performed nutrient enrichment (N and P) and zooplankton exclosure experiments and measured changes in chlorophyll (Chl) as an indicator of the severity of nutrient limitation and grazing intensity. Growth responses were compared with independent measures of P limitation (cell P quotas and phosphatase activity) and grazing intensity (zooplankton densities and inferred community grazing rates).

Methods

Study sites

Experiments were conducted at 12 lakes located within the Adirondack Park in upstate New York and two lakes (Mirror Lake and Cone Pond) located in proximity to the Hubbard Brook Experimental Forest in New Hampshire (Table 1). These sites can be broadly characterized as softwater, oligotrophic lakes of moderate size and depth. Most are headwater, drainage lakes that have been previously included in studies of acidification effects (Linthurst et al. 1986; Kretser et al. 1989; Driscoll and van Dreason 1993). Detailed investigations of plankton communities have been carried out at a subset of these including Mirror Lake (Makarewicz and Likens 1979), Silver Lake (Siegfried 1991), and Woods Lake, Cranberry Pond, and Dart's Lake (Bukaveckas 1989; Bukaveckas and Shaw 1997). The lakes were selected on the basis of pH and acid-neutralizing capacity (ANC) to represent varying stages of lake acidification (Table 1). The group comprised three chronically acidic lakes (pH < 5.0, ANC < 0 μequiv.·L^{-1}), five moderately acidic lakes (pH = 5.0–6.0, ANC = 0–20 μequiv.·L^{-1}), and six circumneutral lakes (pH > 6.0, ANC > 20 μequiv.·L^{-1}). One of the circumneutral lakes (Woods) has been experimentally maintained at this pH by lake and watershed liming (Bukaveckas and Shaw 1997). A second criterion for site selection was dissolved organic carbon (DOC) concentrations. By excluding lakes of DOC > 5 mg·L^{-1}, we eliminated brownwater (colored) lakes where humics and other organic acids contribute substantially to lake acidity. Our intent was to focus on atmospherically acidified lakes, and we consider the issue of naturally acidic (bog influenced) lakes in a related study (Saunders 1992). The primary gradient (lake pH) was significantly correlated with several other variables including total

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and monomeric Al ($R^2 = 0.75$ and 0.54, respectively), Chl ($R^2 = 0.41$), and total dissolved phosphorus (TDP) ($R^2 = 0.27$).

Lake sampling

Forty lake visits were made during the 2-year study to carry out enclosure experiments and collect ancillary data. An attempt was made to visit each lake on at least two occasions (July of 1990 and 1991) and, unless otherwise noted, site data and experimental results are presented as an average for the two visits. Four lakes (Limekiln, Arbutus, McBride, and Wolf) were visited on only one occasion. Two sites (Dart's and Woods) were visited monthly from May to August in both years to compare seasonal and between-site variation in experimental results. Lake data included (*i*) depth profiles of temperature, photosynthetically active radiation, and Chl, (*ii*) an integrated water column sample for chemical analyses (pH, ANC, Al, DOC, nutrients), and (*iii*) vertical zooplankton net tows (80-μm mesh size) taken from 1 m above the bottom to the surface (see Bukaveckas and Shaw 1997).

Enclosure experiments

Nutrient and zooplankton manipulations were performed within 10-L, translucent, polyethylene enclosures ("cubitainers"). The factorial design consisted of 12 enclosures (three replicates each of): controls (no nutrients added), P addition (+P), N addition (+N), and a combined P and N addition (+PN). In some experiments, the +N treatment was omitted. The N and P additions allowed us to identify which nutrient was limiting production and to quantify phytoplankton growth under nutrient-saturated conditions. To ensure that growth would not be nutrient limited during the 48-h incubation, we added 18 μg $P\text{-}PO_4 \cdot L^{-1}$ (as 100 μg $K_2HPO_4 \cdot L^{-1}$) and 138 μg $N\text{-}NO_3 \cdot L^{-1}$ (as 1000 μg $KNO_3 \cdot L^{-1}$) at the start of each experiment. Enclosures were filled from a large (125 L) mixing container after water was pumped from a depth approximating the midpoint of the epilimnion (2–3 m). Macrozooplankton were removed by filtering the water through 100-μm Nitex mesh. Periodic checks of the filtered water revealed that only rotifers had passed through the 100-μm mesh. Passage of the lake water through Nitex did not significantly reduce Chl concentrations based on comparisons of lake samples and initial concentrations in the enclosures. Enclosures were suspended at a depth of 2–3 m to avoid photoinhibition and incubated for 48 h (algal growth on the enclosure walls became apparent after 3 days).

The response to nutrient addition (NR) was calculated as the percent increase in Chl relative to enclosures receiving no nutrients:

$$NR = (Chl_{nutrients} - Chl_{control})/Chl_{control}$$

where $Chl_{nutrients}$ is the mean Chl concentration after 48 h among three replicates receiving nutrient additions (+P, +N, or +PN) and $Chl_{control}$ is the mean Chl concentration after 48 h among replicates receiving no nutrients. NR data were interpreted as follows: single nutrient limitation by P when response to +P and +PN was greater than that observed in control and +N enclosures, single nutrient limitation by N when response to +N and +PN was greater than that observed in control and +P enclosures, and colimitation when response to +PN was greater than that for either N or P alone.

We quantified the response to grazer removal (GR) under both ambient and nutrient-enriched conditions by comparing initial and final Chl concentrations among enclosures with or without added nutrients (all with zooplankton removed):

$$GR_{(amb)} \text{ or } GR_{(+PN)} = (Chl_{final} - Chl_{initial})/Chl_{initial}$$

where $Chl_{initial}$ and Chl_{final} are the mean Chl concentrations of three replicate enclosures (ambient or nutrients added) at the beginning and end of the experiment. The use of true controls (enclosures with zooplankton left intact) is problematic because enclosing daytime surface assemblages would likely underestimate grazing effects in lakes where diel vertical migration occurs.

Estimated grazing

We derived an estimate of community grazing from species abundance data and published species-specific grazing rates. The equations of Sierszen and Frost (1990) were used to estimate grazing rates for dominant herbivorous zooplankton (*Bosmina*, *Daphnia*, and *Diaptomus minutus* adults and copepodites). The equations were derived from measured (in situ) grazing rates for the same taxa in a softwater, oligotrophic lake (Little Rock) in northern Wisconsin. In Little Rock Lake, species-specific grazing rates were unaffected by changes in pH during experimental acidification from 6.2 to 5.2. These equations were applied to data collected from all sites to derive species-specific grazing rates (millilitres per individual per hour) as a function of lake temperature (average water column temperature on dates when zooplankton tows were collected). As Sierszen and Frost (1990) did not measure grazing by *Diaptomus minutus* nauplii, data from Bogdan and Gilbert (1984) were used. Both studies measured feeding rates using *Chlamydomonas* and derived comparable estimates for adults (8.9 and 10.8 $mL \cdot individual^{-1} \cdot day^{-1}$) and copepodites (7.0 and 8.5 $mL \cdot individual^{-1} \cdot day^{-1}$) (Bogdan and Gilbert 1984 and Sierszen and Frost 1990, respectively). For *Diaphanosoma*, we used the grazing rate measured by Bleiwas and Stokes (1990) based on feeding trials using *Chlorella*. Species-specific grazing rates were multiplied by the density of individuals found in the water column on the day of the experiment and expressed as percent of water column filtered per day.

Analytical methods

Chemical analyses included measurements of pH (glass electrode calibrated with low ionic strength buffer solution), ANC (Gran titration), and dissolved oxygen (Winkler titration). Concentrations of chlorophyll *a* (Chl *a*) and phaeophytin were determined spectrophotometrically (Speziale et al. 1984) after filtering through Gelman A/E glass fiber filters (0.45 μm), grinding filters into a slurry, and extracting overnight in aqueous (90%) acetone. Total phosphorus (TP) and total dissolved phosphorus (TDP) concentrations were determined following persulfate oxidation to phosphate of filtered (0.45 μm) and unfiltered samples. Phosphate (ascorbic acid reduction method) and nitrate (Cd reduction method) were determined by autoanalyzer (American Public Health Association 1985). DOC was measured by persulfate digestion followed by IR detection of CO_2. Total and labile monomeric Al were determined using the method developed by Driscoll (1984). Phosphatase activity was measured at 10 sites sampled in 1991 using the method of Boavida and Heath (1984). Phosphatase activity was determined for a range of pHs by incubating samples with 300 μM *p*-nitrophenyl phosphate (pNPP) and 0.3 mL of either acetate–Mg buffer (adjusted to pH 5, 6, and 7) or Tris–Mg buffer (at pH 8 and 9). Distilled water blanks (including substrate and buffer) were run to determine whether non-phosphatase-mediated breakdown of pNPP occurred during the incubation. No correction was required for the breakdown of pNPP, but absorption readings were corrected for interference at 395 nm using zero time blanks.

Results

Seasonal variation

At sites where experiments were conducted monthly during two summers (Woods and Dart's), we did not observe any consistent seasonal patterns in response to nutrient enrichment or removal of macrozooplankton (Fig. 1). In both ambient and nutrient-enriched enclosures ($GR_{(amb)}$ and $GR_{(+PN)}$), removing grazers elicited a stronger response during late-summer experiments but seasonal differences were not statistically significant based on a comparison of group means for May–June and July–August experiments ($N = 15$). We calculated

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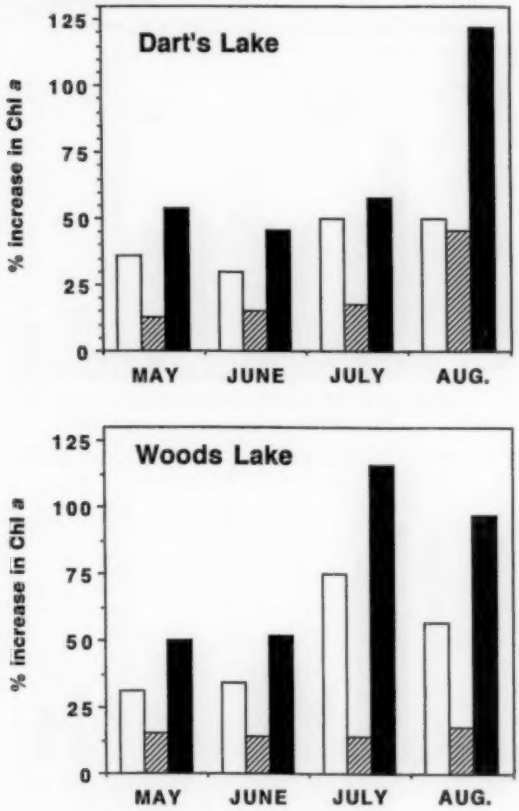
Fig. 1. Seasonal variation in phytoplankton responses to grazer removal (open bars), nutrient addition (hatched bars), and grazer removal/nutrients added (solid bars) at Dart's Lake (pH = 5.1) and Woods Lake (pH = 6.5).



coefficients of variation (CV) based on the mean and standard deviation of $GR_{(amb)}$, $NR_{(+PN)}$, and $GR_{(+PN)}$ for experiments pooled within and among sites (Table 2). For $NR_{(+PN)}$, within-site (temporal) variability at Woods Lake and Dart's Lake (CV = 51 and 59%, respectively) was smaller than that observed among sites (CV = 85%). This finding was consistent with our hypothesis that between-site differences would be important in determining phytoplankton responses to nutrient addition. In contrast, within-site variation in response to grazer removal ($GR_{(amb)}$ and $GR_{(+PN)}$) was comparable with that observed among the 14 lakes (Table 2). In subsequent analyses of nutrient response data, we used a subset of the data (July experiments only) to interpret patterns of variation in relation to lake characteristics (pH and correlated parameters). Phytoplankton responses to grazer removal were interpreted in relation to zooplankton community characteristics using the pooled data set.

Nutrient limitation

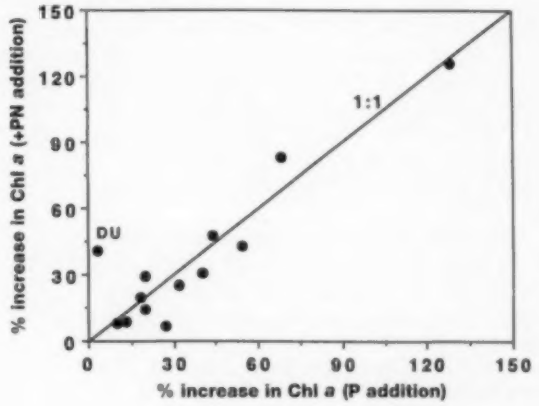
For 13 of the 14 lakes, increases in Chl in the +P and +PN enclosures corresponded closely, suggesting that P was the limiting nutrient (Fig. 2). At one site (Duck), the response to the combined addition (+41%) was substantially greater than

Table 2. Comparison of spatial and temporal variability in response parameters $GR_{(amb)}$ (grazer removal, ambient nutrients), $NR_{(+PN)}$ (nutrient addition), and $GR_{(+PN)}$ (grazer removal and nutrient addition).

	N	Treatment	Mean	SD	CV
Within site					
Woods Lake	8	$GR_{(amb)}$	42	39	92
		$NR_{(+PN)}$	20	10	51
		$GR_{(+PN)}$	70	55	78
Dart's Lake	7	$GR_{(amb)}$	42	17	40
		$NR_{(+PN)}$	26	15	59
		$GR_{(+PN)}$	79	34	43
Among sites	14	$GR_{(amb)}$	63	28	44
		$NR_{(+PN)}$	40	34	85
		$GR_{(+PN)}$	131	92	70

Note: Means represent the average increase in Chl over 48 h among *N* experiments.

Fig. 2. Phytoplankton responses to P addition and combined P and N addition among 14 lakes (DU, Duck Lake). Nutrient responses are the percent increase in Chl *a* over 48 h relative to enclosures receiving no nutrients.



that for P added alone (+3%), suggesting that this site was N or co-limited. Results from nutrient enrichment experiments were consistent with nutrient chemistry data showing that Duck Lake had low dissolved inorganic nitrogen (DIN) (Table 1) and among the lowest N:P ratios (by weight). Only one other site (Mirror) exhibited an N:P < 25 and previous work had shown that phytoplankton were co-limited (Gerhart and Likens 1975). At Mirror Lake, we observed significantly higher responses to the combined addition compared with P added alone ($p < 0.05$ for paired *t*-test of three experiments), although differences were small (mean = 29 versus 20% for $NR_{(+PN)}$ and $NR_{(+P)}$, respectively).

Phytoplankton in acidic lakes (pH ≤ 5.5) exhibited stronger growth responses to nutrient addition than those of circumneutral lakes (Fig. 3). Mean values of $NR_{(+PN)}$ for acidic lakes (75 ± 29%) and circumneutral lakes (15 ± 9%) were significantly different ($p < 0.01$). The largest increases in Chl were observed at Cone Pond (+83%), Silver Lake (+126%), and Limekiln Lake (+83%). Cone Pond and Silver Lake were two

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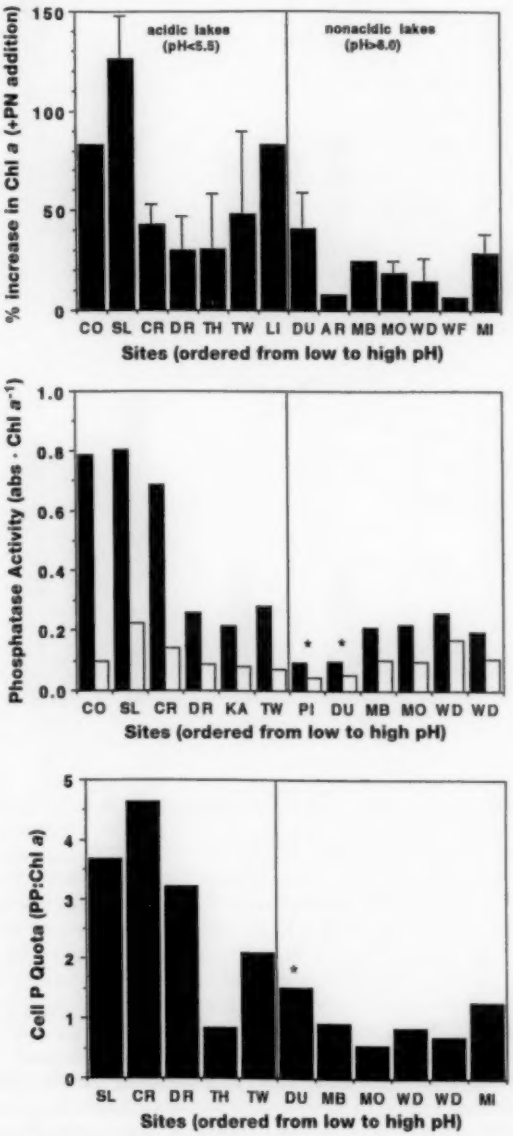
of the three most acidic lakes included in this survey (pH < 5.0) and exhibited the highest concentrations of total and monomeric Al (Al data unavailable for Limekiln). None of the circumneutral lakes exhibited an increase in Chl greater than 30% in response to nutrient addition.

We compared other indicators of nutrient limitation (cell P quotas and phosphatase activity) to determine whether they showed patterns consistent with results from enrichment experiments. Phosphatase data were available for 10 of the 14 lakes included in this study plus two sites (Kanacto, Pitchfork) where nutrient enrichment experiments were conducted as part of a related study (Saunders 1992). These data were included because one of the lakes (Pitchfork) was shown to be co-limited or N limited based on enrichment experiments. Alkaline phosphatase activities measured at pH 7, 8, and 9 were uniformly low at all sites (Fig. 3). Acid phosphatase activities measured at pH 5 and 6 were higher and exhibited an eightfold range of variation among lakes (Fig. 3). Biomass-specific acid phosphatase activity levels were similar (~0.2 absorption units (abs)·μg Chl^{-1}) among the moderately acidified and circumneutral lakes with the exception of two N-limited/co-limited lakes (Pitchfork, Duck) where levels were 50% lower. For this subset of lakes (excluding N-limited sites), there was a strong linear relationship between enzyme activity and Chl (R^2 = 0.92, p = 0.0004). Acid phosphatase activity was substantially higher (0.7–0.8 abs·μg Chl^{-1}) at the three most acidic lakes (Cone, Silver, Cranberry). These sites exhibited the highest Al concentrations, and therefore, phosphatase activity was positively correlated with total and monomeric Al (R^2 = 0.86 and 0.73, respectively). As these sites also exhibited strong responses to nutrient enrichment, phosphatase activity was found to be significantly correlated with $NR_{(+PN)}$ (R^2 = 0.52, p < 0.0001).

Cell quotas (P content per unit of algal biomass) were estimated from the ratio of particulate phosphorus (PP = TP – TDP) to Chl (Q = micrograms PP per microgram Chl). Cell P quotas below 1 μg PP·μg Chl^{-1} have been shown to initiate a response in phosphatase activity (Connors et al. 1996). In this study, cell P quotas ranged from <1 to 5 μg PP·μg Chl^{-1} and were highest among the most acidic lakes (Fig. 3). Cell P quotas were weakly correlated with responses to P addition and phosphatase activity (R^2 < 0.30).

We used regression analyses to relate between-site variation in nutrient response to a range of lake characteristics. Lake pH was negatively correlated with nutrient response but explained only 20% of the variation (p = 0.035) whereas monomeric Al concentrations were positively correlated and accounted for a greater proportion of the variation (R^2 = 0.45, p = 0.0008). The latter was due to the fact that two of the sites exhibiting strong responses to nutrient enrichment (Cone, Silver) had the highest concentrations of total and monomeric Al. The percentage of subsurface irradiance reaching the depth where enclosures were incubated was found to be the best predictor of $NR_{(+PN)}$ (R^2 = 0.63, p < 0.0001). Enclosures were suspended at depths approximating the midpoint of the epilimnion (typically 3 m) and associated irradiances ranged from 11 to 58%. The three lakes exhibiting the strongest responses to nutrient enrichment (Cone, Silver, Limekiln) had the lowest light attenuation coefficients (kd < 0.30 m^{-1}; Table 1). Lake temperature was not found to be a significant predictor of $NR_{(+PN)}$. Most experiments were conducted in July or August

Fig. 3. Phytoplankton response to combined P and N addition (top panel) (error bars denote SD based on replicate experiments), biomass-specific enzyme activity of acid (pH = 5; solid bars) and alkaline (pH = 8; open bars) phosphatase (middle panel), and cell P quotas estimated from the ratio of PP to Chl *a* (bottom panel) (asterisks denote N-limited lakes). See Table 1 for lake abbreviations.



(26 of 38) when there was little variation in epilimnetic temperatures among sites. None of the indicators used to represent differences in lake trophic state (TDP, epilimnetic Chl, cell P quota) accounted for a significant proportion of the variance in $NR_{(+PN)}$ in univariate or multivariate models.

Grazer effects

Phytoplankton responses to grazer removal were generally

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Fig. 4. Phytoplankton responses to removal of macrozooplankton under ambient nutrient (top panel) and nutrient-saturated (bottom panel) conditions. Grazer responses are the percent increase in Chl *a* over 48 h. See Table 1 for lake abbreviations.

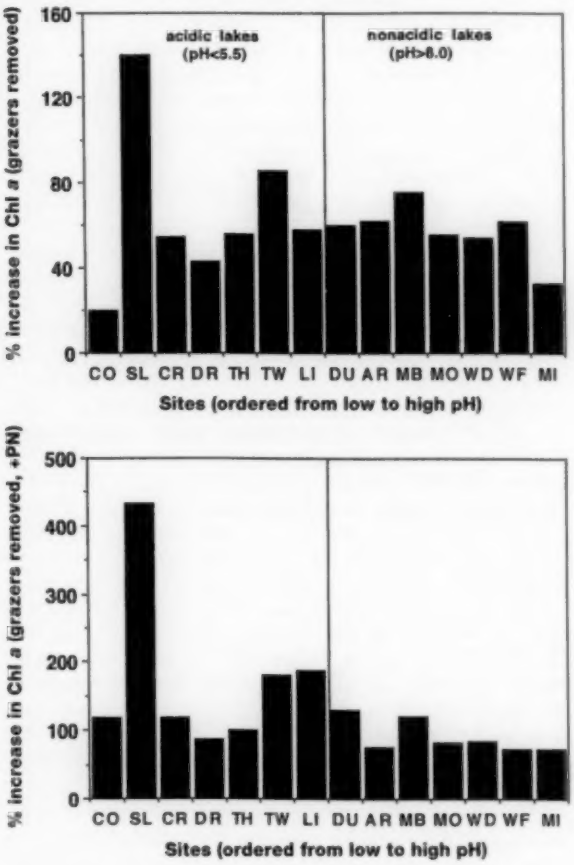
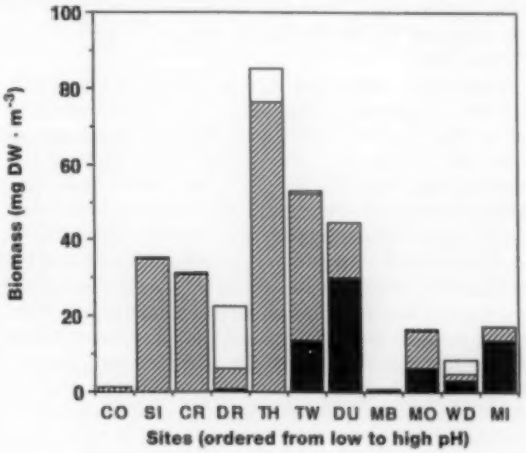

% increase in Chl a (grazers removed)
acidic lakes (pH<5.5)
nonacidic lakes (pH>6.0)
CO SL CR DR TH TW LI DU AR MB MO WD WF MI
Sites (ordered from low to high pH)
% increase in Chl a (grazers removed, +PN)
CO SL CR DR TH TW LI DU AR MB MO WD WF MI
Sites (ordered from low to high pH)

Fig. 5. Biomass (dry weight (DW)) of *Daphnia pulex* (solid bars), *Bosmina longirostris* (open bars), and *Diaptomus minutus* (adults and copepodites) (hatched bars) on dates when macrozooplankton exclosure experiments were conducted. See Table 1 for lake abbreviations.



similar among acidic and non-acidic lakes (Fig. 4). Chl concentrations increased by an average of 63% and 131% ($GR_{(amb)}$ and $GR_{(+PN)}$, respectively) during the 48 hr experiments. Neither of the grazing response parameters were significantly correlated with lake pH or aluminum concentrations. Lake macrozooplankton communities were dominated by *Daphnia pulex*, *Bosmina longirostris*, and *D. minutus* (Fig. 5). These three taxa (including nauplii) accounted for >90% of total herbivore biomass (adult cyclopoids excluded) at all sites with the exception of Cone Pond where *Diaphanosoma birgei* was the dominant cladoceran. *Daphnia* were more abundant in the higher pH lakes (5.4–6.8) and *Daphnia* biomass (log-transformed) was significantly correlated with lake pH (R^2 = 0.51, $p = 0.013$). However, total zooplankton biomass was not significantly correlated with lake acidity, as *Bosmina* and *Diaptomus* were more abundant among the low-pH lakes (4.8–5.4).

We analyzed variation in phytoplankton responses to grazer removal in relation to zooplankton densities, biomass, and estimated community grazing rates. Increases in Chl concentrations following grazer removal were significantly and positively correlated with log-transformed zooplankton densities ($R^2 = 0.44$, $p = 0.007$) and biomass ($R^2 = 0.39$, $p = 0.012$). At sites where zooplankton abundance was low (<10 000 individuals·m^{-3}), we observed weak growth responses following removal (10–40% increase in Chl). Among sites where abundance was higher, estimates of $GR_{(amb)}$ ranged from 30 to 100%. The use of a nonlinear (tangential) function substantially improved the fit of both models ($R^2 = 0.84$ and 0.76 for density and biomass, respectively; Fig. 6). Estimated community grazing rates (percentage of water column filtered per day) were also significantly correlated with $GR_{(amb)}$ (nonlinear regression, $R^2 = 0.76$). The use of a tangential model implies that phytoplankton growth responses to grazer removal become saturated at very high grazer densities. However, with few lakes in the high density range, we cannot resolve whether the linear or nonlinear model is the more accurate depiction of this relationship.

Discussion

Our study shows that phytoplankton communities in clearwater (low DOC) Adirondack lakes were predominantly P limited (12 of 14 sites surveyed). This finding is supported by results from nutrient enrichment experiments, assays of phosphatase activity, and epilimnetic N:P ratios. Estimates of cell P quotas (PP:Chl) were not consistent with other indices of nutrient limitation because quotas were highest among lakes exhibiting strong responses to P addition and high phosphatase activity. This estimate is an approximation because the cellular content of Chl is variable and the PP pool also includes bacterial and detrital P. In these lakes where P and Chl concentrations were near detection limits, their ratios may be unreliable indicators of per capita P availability.

P limitation among clearwater Adirondack lakes can be attributed to watershed-scale and regional processes regulating N and P inputs. P export from undisturbed, forested watersheds is low, and data collected in proximity to one of our study sites

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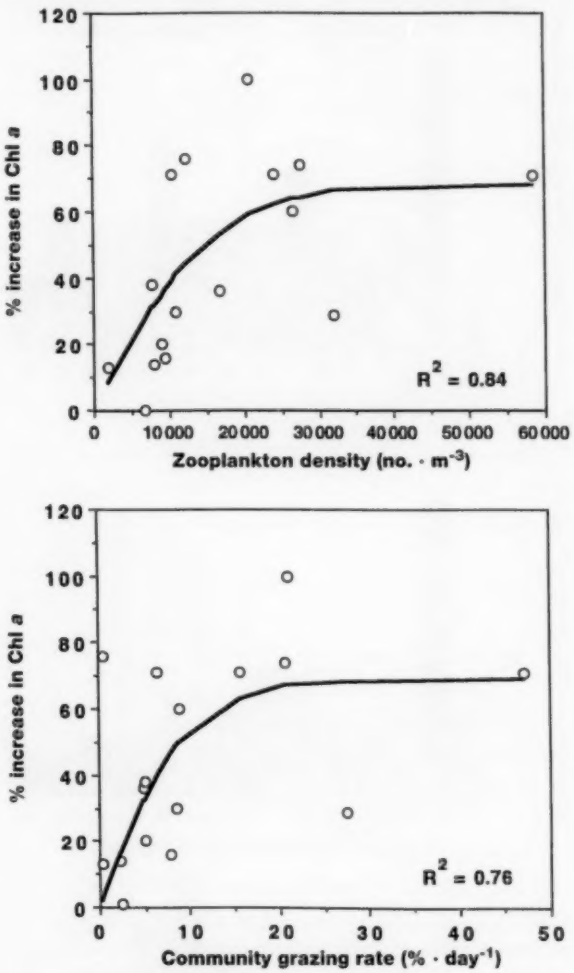
(Arbutus) indicate that leaching of TP from the soil was only 26% of the input from precipitation (Zhang and Mitchell 1995). Atmospheric inputs in this region are elevated in N, resulting in substantial nitrate export to surface waters (Driscoll et al. 1991). The influence of these processes is reflected in the low dissolved P concentrations (mean = 8 $\mu g \cdot L^{-1}$) and high inorganic N concentrations (mean = 257 $\mu g \cdot L^{-1}$) among the study sites.

Atmospheric deposition is potentially a major source of available N supporting algal production in Adirondack lakes and in other regions experiencing elevated deposition of N (Paerl 1985; Axler et al. 1994). Four lakes exhibited low N concentrations (DIN < 100 $\mu g \cdot L^{-1}$), and two of these (Duck, Mirror) were found to be N-limited or colimited based on enclosure experiments and phosphatase activity. These sites were not distinguished by any other physical–chemical attributes, and therefore, we can only speculate as to the mechanisms that may account for N limitation. Mirror Lake has experienced modest shoreline development, and long-term water chemistry data suggest that P inputs to the lake increased during the 1970s–1980s (P. Bukaveckas and G. Likens, unpublished data). Coincident with the increase in P, concentrations of nitrate and dissolved Si have decreased. Duck Lake is large (137 ha) but relatively shallow (mean depth <1 m) compared with other lakes in the survey group. The low lake volume to sediment area ratio accentuates the importance of sediment processes (denitrification, benthic algal assimilation) in regulating water column DIN. Axler and Reuter (1996) have shown that epibenthic algae can account for a substantial proportion of inorganic N assimilation.

Phytoplankton in acidic lakes (pH < 5.0) responded more strongly to nutrient enrichment and exhibited fourfold higher biomass-specific phosphatase activity compared with phytoplankton in nonacidic lakes. These data support the hypothesis that phytoplankton in clearwater (nonhumic), acidic lakes are severely nutrient limited, but it is unclear whether this condition can be attributed to the effects of anthropogenic acidification. It has been suggested that in acidic lakes, P complexes with Al, decreasing its availability for phytoplankton uptake (Broberg and Persson 1984). The presence of monomeric Al in acidified lakes may also interfere with the functioning of phosphatase enzymes and thereby impede phytoplankton uptake of organic P (Jansson et al. 1986). Among lakes in this survey, monomeric Al concentrations were found to be significantly correlated with the severity of P limitation as measured by responses to nutrient enrichment and phosphatase activity. However, epilimnetic TDP was only weakly correlated with monomeric Al, and phosphatase activity was higher, not lower, in the presence of elevated Al. Thus, our data are in agreement with the review by Olsson and Pettersson (1993) concluding that there is little evidence to support the hypothesis that elevated Al concentrations diminish P availability in acidified lakes.

Our experiments suggest that high light availability in acidic lakes allowed phytoplankton to utilize added P more rapidly. At the depths where enclosures were incubated, light availability was higher among acidic lakes, facilitating more rapid production of new biomass under nutrient-saturated conditions. Data from this and other studies (Schofield 1972; P. Bukaveckas and M. Robbins, unpublished data) have shown that acidic lakes (excluding bog waters) have lower light

Fig. 6. Phytoplankton responses to removal of macrozooplankton as a function of zooplankton density (top panel) and inferred community grazing rates (bottom panel). Community grazing rates are expressed as percentage of the water column filtered per day.



attenuation compared with nonacidic lakes in this region. Furthermore, several whole-lake manipulation experiments have documented decreases in light attenuation associated with acidification (Shearer et al. 1987) and increases following neutralization (Bukaveckas and Driscoll 1991). Almer et al. (1978) proposed that coagulation and removal of DOC by Al may account for increased light availability in acidified lakes. Data collected by Effler et al. (1985) at one of the sites included in this survey (Dart's) support this hypothesis. They showed that seasonal changes in light attenuation corresponded to fluctuations in DOC that were coupled with the loss of Al from the water column.

Differences in transparency among lakes of varying acidity may in part account for the observed patterns in phytoplankton responses to nutrient enrichment, but it is problematic to infer historical trends from the spatial patterns. Lakes that are

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sensitive to acidification by atmospheric inputs are characterized by watershed drainage patterns in which there is little opportunity for soil–water interactions to neutralize the acidity of runoff (Goldstein et al. 1985). In these watersheds, the export of nutrients, DOC, and particulate matter is low, resulting in clearwater and oligotrophic conditions. We cannot discount the possibility that lakes that are prone to acidification may have historically exhibited greater transparency and lower nutrient availability compared with other lakes in the region.

Phytoplankton responses to the removal of macrozooplankton did not exhibit any consistent patterns among lakes that differed in their acidity and zooplankton community composition. Our expectation that phytoplankton responses to macrozooplankton removal would be greater where *Daphnia* were abundant was based on two lines of evidence: (*i*) biomanipulation and cascading trophic interaction hypotheses predict that an increase in the biomass of large zooplankton from reduced planktivory should result in higher grazing rates and reduce phytoplankton biomass (Shapiro and Wright 1984; Carpenter et al. 1985) and (*ii*) feeding trials have shown that copepods feed at lower rates than cladocerans and that cladoceran feeding rates increase with body length (Peters and Downing 1984; Chow-Fraser and Knoechel 1985; Knoechel and Holtby 1986). We expected stronger responses among nonacidic lakes where large herbivores (*Daphnia*) were more abundant. A similar range of responses was observed among *Daphnia*-dominated lakes and acidic lakes that were dominated by smaller species (principally *Diaptomus minutus* and *Bosmina longirostris*). Our data support the findings of Cyr and Pace (1992) that communities dominated by large zooplankton do not exhibit higher grazing rates than those dominated by small zooplankton. Results from our experiments were also consistent with those of Sierszen and Frost (1990) indicating that zooplankton community grazing rates were not substantially affected by acidification.

In our experiments, we measured the increase in Chl as an indicator of the intensity of herbivory. Other factors such as nutrient availability will constrain phytoplankton growth responses following grazer removal and may account for some of the variability in our experiments. A number of studies have demonstrated that phytoplankton are variously dependent on nutrients recycled by zooplankton (Sterner et al. 1995). Removing zooplankton from enclosures may increase the severity of nutrient limitation. We did not find that lake nutrient concentrations were a significant predictor of phytoplankton response to macrozooplankton removal at ambient nutrient levels in either univariate or multivariate (with zooplankton density or biomass) regressions. Furthermore, a comparison of phytoplankton responses to grazer removal among ambient and nutrient-enriched enclosures did not reveal any consistent differences with respect to lake acidity or community composition. We did find that zooplankton abundance at the time of the experiment was a significant predictor of phytoplankton responses to grazer removal. A tangential model explained 84% of the variation among experiments conducted at the 14 sites and supports our contention that the response variable is reflective of grazing intensity.

In summary, we used field experiments to measure phytoplankton responses to nutrient addition and macrozooplankton removal along a gradient represented by 14 lakes at varying stages of acidification. Responses to nutrient addition varied in a predictable fashion based on ambient light availability, and responses to zooplankton removal showed a nonlinear relationship with zooplankton density. Our results indicate that manipulation experiments can be useful tools in the context of comparative lake surveys and impact assessment. In a related study, we used a similar approach to compare anthropogenically acidified lakes with naturally acidic (bog) waters and showed that patterns of nutrient limitation are related to lake hydrology (Saunders 1992).

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Effects of wave disturbance and substrate slope on sediment characteristics in the littoral zone of small lakes

Hélène Cyr

Abstract: Sediment characteristics in the littoral zone of lakes affect the distribution, biomass, and productivity of benthic organisms and affect chemical exchanges with the water column, yet we know very little about their distribution. I tested whether sediment water and organic matter content were related to site exposure and to basin morphology by comparing sediments along 12 transects in three small lakes (area <150 ha). The distribution of sediment water content in small lakes differed from the general patterns described for large lakes and ocean. Sediment water content was generally high in shallow waters (<2–5 m), decreased sharply to 30–50% within 20 m from shore, and increased again in the deeper portion of the littoral zone. Fine sediments were retained in the shallow littoral zone (<1.5 m) at sites with mean fetch <150 m. The maximum depth at which sediments were mixed increased with increasing depth of the wave-mixed layer and with increasing slope of the substrate. The organic matter content (OM) of littoral sediments was closely related to their water content (WC) ($\log(OM) = -4.7 + 3.0 \log(WC)$, $r^2 = 0.77$, $P < 0.001$), and sediment organic content was very similar in the upper and lower portions of the littoral zone.

Résumé : Les sédiments de la zone littorale des lacs influent sur la distribution, l'abondance et la productivité des organismes benthiques ainsi que sur les échanges chimiques survenant dans la colonne d'eau; pourtant, nous connaissons très mal leur distribution. J'ai vérifié si la teneur en eau et la teneur en matières organiques des sédiments sont liées à l'exposition de la région considérée et à la morphologie du bassin lacustre, en comparant les sédiments sur 12 transects délimités dans trois petits lacs (d'une étendue de <150 ha). La distribution des valeurs de la teneur en eau des sédiments des petits lacs diffère de la distribution générale établie pour les lacs étendus et pour l'océan. En général, cette valeur était élevée dans les zones d'eau peu profonde (moins de 2–5 m) et baissait à 30–50% de cette valeur dans un rayon de 20 m de la rive, pour s'élever à nouveau dans les parties plus profondes de la zone littorale. Les sédiments fins étaient retenus dans la zone littorale peu profonde (<1,5 m) à des points où le fetch moyen était inférieur à 150 m. La profondeur maximale de la couche où les sédiments étaient mélangés augmentait en fonction de la profondeur de la couche mélangée sous l'action des vagues et en fonction de la pente du substrat. Quant à la teneur en matières organiques (MO) des sédiments de la zone littorale, elle était étroitement liée à la teneur en eau (TE) [$\log(MO) = 4{,}7 + 3{,}0 \log(TE)$, $r^2 = 0{,}77$, $P < 0{,}001$], et la valeur mesurée dans le haut de la zone littorale était très proche de celle du bas.
[Traduit par la Rédaction]

Introduction

The physical and chemical composition of sediments in different parts of aquatic systems depends on the relative importance of different sources of sediment particles (e.g., inflow from streams and rivers, settled plankton) and on physical processes that redistribute these particles (e.g., waves, currents; Sly 1978; Håkanson and Jansson 1983; Rowan et al. 1992). The frequency and intensity of sediment disturbances vary spatially with depth, site exposure, and substrate morphology and temporally (hourly, seasonally) with meterological conditions and thermal stratification. Sediment disturbances and redistribution affect chemical exchanges (e.g., nutrients, contaminants) between sediments and the water column (e.g., Burkholder 1992) and determine the conditions under which benthic organisms live in different parts of the lake and at different times of the year.

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Most research on sediment distribution has focused either on offshore sediments, because of their importance as repositories of contaminants (e.g., Håkanson 1977; Rowan et al. 1992; Blais and Kalff 1995; Stephenson et al. 1995) and of paleoindicators (e.g., Davis 1989; Smol 1992), or on inshore sediments in oceans and large lakes to study beach erosion and shoreline movements (e.g., Komar 1976; U.S. Army 1984). The distribution of sediment characteristics in the littoral zone of lakes is poorly known, especially in small lakes. Yet, from a biological standpoint, the littoral sediments should be the most important, since they are located in the warmest, most structured, and, per unit area, most productive part of aquatic ecosystems (Wetzel 1990). Sediment characteristics (e.g., particle size, organic content) are closely related to the distribution, biomass, and productivity of benthic organisms (e.g., Jónasson 1978; Cyr and Downing 1988; Schallenberg and Kalff 1993).

Sediment distribution is largely determined by wave action and substrate morphology (Håkanson and Jansson 1983; Hilton 1985; Rowan et al. 1992). Direct wave action can resuspend sediments several metres below the water surface, depending on site exposure, wind speed, and wind duration (Smith and Sinclair 1972; Komar 1976; U.S. Army 1984). In

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unstratified shallow aquatic systems, where wind energy is continually transferred to the bottom through wave action, the sediments are resuspended and redistributed randomly throughout the system year-round (Davis 1973; Carper and Bachmann 1984; Demers et al. 1987). Clearer patterns of sediment distribution have been observed in deeper stratified aquatic systems, where sediments are only resuspended from shallow areas above the thermocline, and during periods of destratification. Particle sorting occurs in relatively deep systems because large resuspended particles settle quickly, while fine particles remain in suspension longer and are transported gradually to greater depths where insufficient energy is available for resuspension. This process is called sediment focusing and results in a systematic decline in sediment particle size with increasing depth (Sly 1978). The higher the wind energy, the larger the particles that can be transported and the steeper the depth gradient in particle size distribution (Sly 1978). Since sediment water content is inversely related to sediment particle size (Håkanson 1977; Rowan et al. 1992), the water content of sediments generally increases with increasing depth (e.g., Håkanson 1977; Blais and Kalff 1995; Stephenson et al. 1995). The bottom of lakes and oceans is divided into an inshore zone of erosion where sediments are constantly resuspended by wave action and have low water content, transportation zones where sediments are deposited temporarily and have intermediate water content, and accumulation zones where sediments are deposited permanently and have high water content (Håkanson 1977; Rowan et al. 1992).

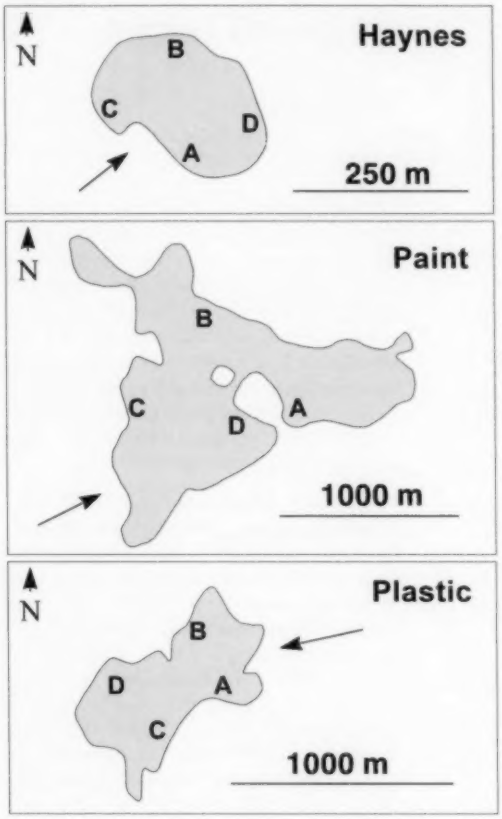
Substrate morphology is also known to affect sediment distribution (Håkanson 1977; Hilton 1985; Rowan et al. 1992; Blais and Kalff 1995). Sediment particles deposited along steeper slopes are less stable, increasing the probability of sediment slumping and sliding (Håkanson and Jansson 1983; Hilton et al. 1986). Several studies report that slopes steeper than 3–5% have a significant effect on sediment distribution (Håkanson 1977; Rowan et al. 1992; Blais and Kalff 1995). Models developed by Rowan et al. (1992) suggest an exponential relationship between the depth at which fine sediments can get deposited in large lakes and slope of the substrate. These models remain to be tested in smaller lakes and on steeper slopes.

In this study, I test whether sediment characteristics in the littoral zone of small lakes, with maximum fetch <1 km, are related to site exposure and to basin morphology by comparing sediment water and organic content along 12 transects in three oligo-mesotrophic lakes. The littoral zone is defined as the area where more than 1% of the incident radiation penetrates to the bottom of the lake. I also test whether models developed by Rowan et al. (1992) to predict the depth of the mud deposition boundary in large lakes (surface area >10 km^2) can be extrapolated to small lakes and to steeper slopes.

Methods

The sediments were sampled in three oligo-mesotrophic lakes of southern Ontario, Canada, between May and August 1995 (Fig. 1; Table 1). Haynes is a small mesotrophic kettle lake located on the Oak Ridges Moraine, 30 km north of Toronto (43°57′N, 79°25′W, area = 2 ha, maximum depth = 16.0 m, mean depth = 6.9 m). Paint and Plastic are oligotrophic lakes located on the Precambrian Shield (Paint: 45°13′N, 78°56′W, area = 145 ha, maximum depth = 21.4 m, mean depth = 6.9 m; Plastic: 45°11′N, 78°50′W, area = 32 ha, maximum depth = 16.3 m, mean depth = 7.9 m).

Fig. 1. Lake outlines with the position of four transects (A–D) where sediments were sampled. Long arrows indicate the mean wind direction during each sampling period (sampling dates are listed in Table 1). Note differences in scale.



Sediment cores were collected along twelve 25- to 50-m transects that extended from shore to the metalimnion or upper hypolimnion of the three lakes. All samples were collected within the oxygenated (dissolved oxygen >6 $mg \cdot L^{-1}$) euphotic (>4% surface irradiance) zone of each lake. The transects were located at sites that varied sevenfold in mean fetch (82–558 m) along substrates with shallow to very steep slopes (4–40%) (Fig. 1; Table 1).

Metered transects were stretched perpendicular to shore at four sites around each lake, covering as broad a range of site exposure as possible (Fig. 1). Rings of 10-cm-diameter PVC pipe were dropped from the lake surface at 0.5- to 1-m intervals along each transect. These rings settled very gently to the bottom without disturbing the sediments and were used to distribute the sediment samples along each transect. SCUBA divers collected sediment samples from the centre of each ring using 5.2-cm corers and recorded the depth at which each sample was collected using a wrist depth gauge (Digital, ±0.3 m). Each core was carefully brought back to the boat, where the surface 5 cm of sediments was extruded with a piston, sliced, and kept on ice until return to the laboratory. The samples were weighed, homogenized, and two or three replicate subsamples dried at 60°C for 2 days to determine water content (as a percentage of wet mass) and ashed at 550°C for 2.5 h to determine organic matter content (as a percentage of dry mass).

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Temperature and oxygen concentration were measured from the boat at 1-m depth intervals through the water column using a YSI meter (model 57). Mean slope of the substrate was calculated from all depth readings using simple linear regression analysis and is expressed as percent depth increase over a standard horizontal distance (Håkanson and Jansson 1983). Fetch (f, metres) was calculated as the weighed average distance over open water (i.e., distance between a site and land or an island across the lake) in a given direction (Håkansson and Jansson 1983):

$$f = \left(\sum x_i \cdot \cos \gamma_i\right) \Big/ \left(\sum \cos \gamma_i\right)$$

where x_i is the distance over open water (metres) at angle i from the direction being measured and γ_i are 15 angles from a given direction over which x_i is measured (0, ±6, ±12, ±18, ±24, ±30, ±36, and ±42°). All fetches were measured using 1 : 50 000 topographical maps. Maximum and mean fetch were calculated from fetch measured in five directions from shore (0, 45, 90, 135, and 180°). Effective fetch was measured in the mean direction of the wind during each sampling period (calculated from hourly wind measurements; Fig. 1). Wind data used for Paint and Plastic lakes were collected on the shores of Paint Lake, <10 km from Plastic Lake, by the Ontario Ministry of Environment and Energy, Dorset, Ont. Wind data used for Haynes Lake were collected about 12 km south of the lake, at the Buttonville Airport, Markham, Ont. Effective fetches of 0 m were calculated at sites where winds were blowing offshore.

Rowan et al. (1992) proposed two closely related models to predict the depth of mud deposition boundaries (MDB) in large lakes. I tested whether their model based on maximum fetch and slope of the substrate (eq. 25 in Rowan et al. 1992) extrapolates to small lakes by comparing depths of MDB predicted from their model with those observed in Haynes, Paint, and Plastic lakes. The depth of MDB was estimated by fitting a moving-average model (LOWESS, Cleveland 1979) to each profile of sediment water content (Figs. 2–4) and determining the depth where sediment water content increased to 60% (definition of MDB used by Rowan et al. 1992). For transects where sediment water content decreased below 75%, but did not increase again (i.e., transects HAY-D and PNT-B; Figs. 2 and 3), the maximum sampling depth was used as a minimum estimate of the MDB depth.

The depth of the wave-mixed layer was estimated in two ways. First, wave period (T, seconds) was predicted from mean fetch (F, metres) and from wind speed (w, metres per second) using the empirical model developed by the Beach Erosion Board (Smith and Sinclair 1972):

$$(1) \qquad g \cdot T/w = 0.46(g \cdot F/w^2)^{0.28}$$

where g is the gravitational constant (9.8 m·s^{-2}). Wave length (L, metres) was then calculated as (Smith and Sinclair 1972)

$$(2) \qquad L = 1.56T^2.$$

The maximum wave length at a site was estimated using wind speed expected during a large storm (w = 19 m·s^{-1}; Smith and Sinclair 1972). The depth of the wave-mixed layer (h, metres) was estimated as 1/2(L) (Smith and Sinclair 1972; U.S. Army 1984) by solving eq. 1 for T and substituting it into eq. 2 and setting w = 19 m·s^{-1}:

$$(3) \qquad h_{L/2} = 0.082F^{0.56}.$$

Wave periods predicted from the Beach Erosion Board model (eq. 1) are slightly lower, but closely correlated with wave periods predicted using more recent models (e.g., Donelan 1980, $r^2 = 0.89$), suggesting that the wave-mixed layer could be 0.5–1.5 m deeper than $h_{L/2}$. These estimates of the wave-mixed layer assume that all particles within one half the wave length will be affected by the waves, regardless of their size or density.

A second approach is to calculate the depth where the threshold of sediment movement is reached for a given particle size and density (Komar 1976). The threshold velocity (U_t, metres per second) needed to resuspend small sediment particles (<500 µm in diameter) is calculated using

$$(4) \qquad \frac{\rho \cdot U_t^2}{(\rho_s - \rho) \cdot g \cdot D} = 0.21(d_o / D)^{0.5}$$

where ρ is water density (1.0 g·cm^{-3}), ρ_s is sediment particle density (grams per cubic centimetre), D is sediment particle diameter (metres), and d_o is orbital diameter (metres; Komar 1976). The threshold velocity for 23-µm sediment particles ($D = 23 \times 10^{-6}$ m, equivalent to 60% water content; Rowan et al. 1992) was calculated using ρ_s = 1.3 g·cm^{-3}, a value calculated according to Håkanson (1977, eq. 4), which is more appropriate for loosely consolidated organic littoral sediments than the value for quartz sand (ρ_s = 2.65 g·cm^{-3}) used by Komar (1976) and Rowan et al. (1992). The value of ρ_s used here is within the range of sediment particle densities used to develop the model (1.3–7.9 g·cm^{-3}; Komar and Miller 1973), but the model was developed for larger particles (90–8000 µm). Particle size analysis at a few sites suggest that the sediments along PLA-B and HAY-B were fine silt ($D = 10–13 \times 10^{-6}$ m; H. Cyr, unpublished). Calculations with $D = 23 \times 10^{-6}$ m, therefore, are more representative of the sediments sampled in this study and can be compared with the model of Rowan et al. (1992). Near-bottom orbital diameter (d_o) is calculated at a given depth (h) from wave height (H, metres) and wave length (L) according to (Komar 1976; Rowan et al. 1992)

$$(5) \qquad d_o = \frac{H}{\sinh(2\pi h/L)}.$$

Wave height (H, metres) was predicted from mean fetch (F, metres) and from wind speed (w, metres per second) using the empirical model developed by the Beach Erosion Board (Smith and Sinclair 1972):

$$(6) \qquad g \cdot H/w^2 = 0.0026(g \cdot F/w^2)^{0.47}.$$

Substituting eq. 5 into eq. 4, substituting fetch (F) for wave length (L) and wave height (H) using eqs. 1, 2, and 6, and setting w = 19 m·s^{-1}, the near-bottom threshold velocity for 23-µm sediment particles becomes

$$(7) \qquad U_{t23} = \frac{0.0198F^{0.1175}}{[\sinh(38.2 \cdot h \cdot F^{-0.56})]^{0.25}}.$$

By comparing the threshold velocity for 23-µm sediment particles (U_{t23}) with the maximum horizontal velocity at the bottom of a wave

Table 1. Characteristics of sampling sites.

Transect	Date	n_{cores}	Mean fetch (m)	Effective fetch (m)	Substrate slope (%)
HAY-A	May 26	49	90	0	26
HAY-B	May 30	48	95	106	40
HAY-C	June 1	43	82	0	33
HAY-D	June 5	48	89	129	26
PNT-A	July 15	49	225	334	4
PNT-B	July 20	24	558	329	30
PNT-C	July 21	44	474	0	8
PNT-D	July 22	39	482	445	14
PLA-A	August 8	48	216	67	16
PLA-B	August 9	48	251	303	14
PLA-C	August 10	45	310	0	9
PLA-D	August 10	47	266	399	14

Note: Cores were collected along four transects (A–D) in each of three lakes (HAY, Haynes Lake; PNT, Paint Lake; PLA, Plastic Lake; see Fig. 1). Sampling date, number of cores where sediment characteristics could be measured (n_{cores}), mean fetch, effective fetch, and substrate slope are reported for each transect.

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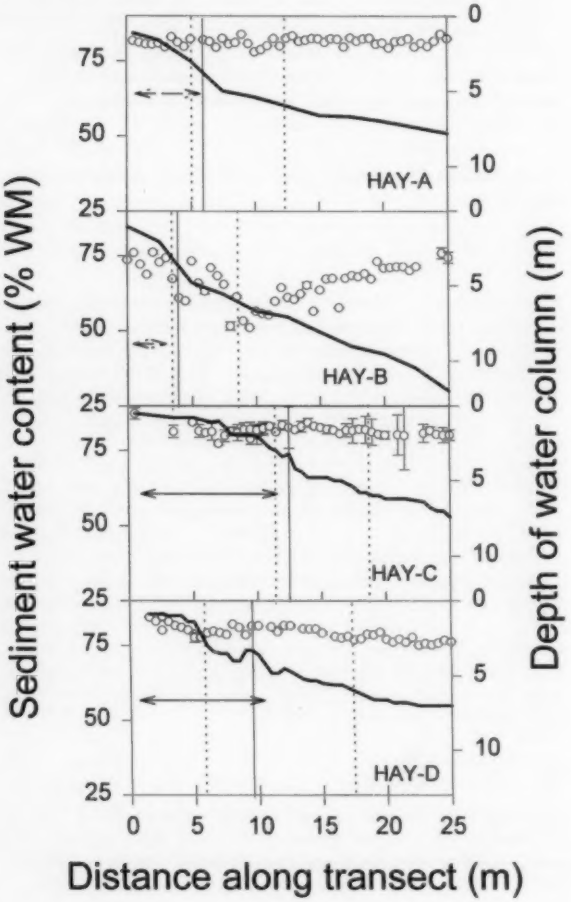
Fig. 2. Depth profiles of the water column and sediment water content (WM, wet mass) along four transects (A–D) in Haynes Lake. Site characteristics are described in Table 1 and Fig. 1. Samples were collected at 0.5-m intervals along 25-m transects and range down to 7- to 12-m depths depending on bottom morphology. Thick solid lines indicate the depth of the water column along each transect. Circles represent the average water content from two or three replicate subsamples. Error bars are standard errors and are often too small to be seen. Solid vertical lines represent the depth of the thermocline (3.5 m) and broken vertical lines represent the top and the bottom of the metalimnion. Horizontal arrows indicate the extent of macrophyte beds (mostly *Myriophyllum*). Broken arrows in HAY-A and HAY-B indicate the approximate location of the macrophyte beds, since this was not recorded during sampling.

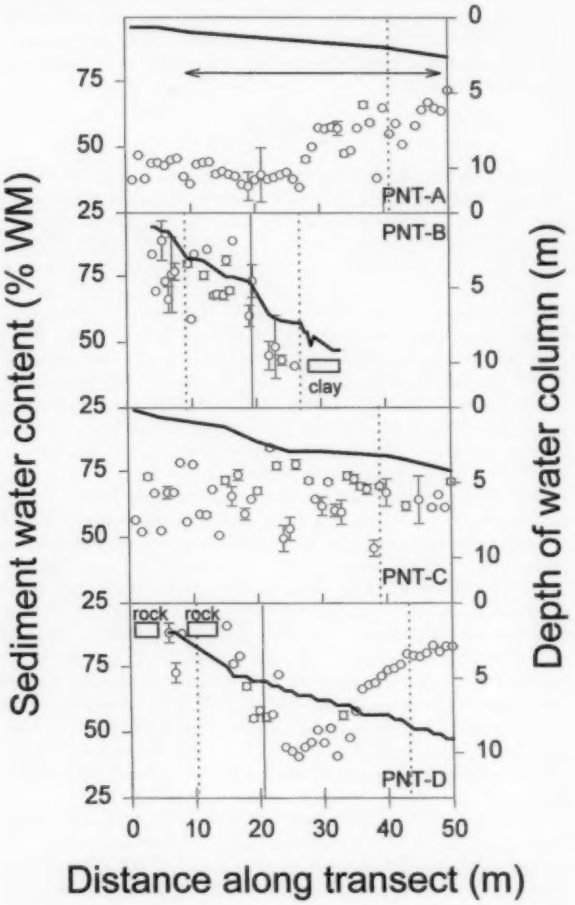


(U_m), we can determine whether or not the particles will be resuspended by the wave; U_m is calculated as (Komar 1976)

$$(8) \quad U_m = \frac{\pi H}{T \sinh(2\pi h/L)}$$

Wave height (H), period (T), and length (L) in eq. 8 can be expressed in terms of fetch (F) using eqs. 1, 2, and 6, and by setting $w = 19$ m·s^{-1}, U_m can be calculated as

Fig. 3. Same as Fig. 2 in Paint Lake. Boxes indicate the locations where cores could not be collected (i.e., rock or clay substrate). The horizontal arrow along transect A indicates the extent of a macrophyte bed (*Isoetes* and sparse *Potamogeton* spp.).



$$(9) \quad U_m = \frac{0.170F^{0.19}}{\sinh(38.2 \cdot h \cdot F^{-0.56})}$$

The depth at which 23-μm sediment particles will be resuspended can be determined for sites with different fetch by equating eqs. 7 and 9 (i.e., $U_{t23} = U_m$), and solving for h:

$$(10) \quad h_{U_{t23}} = 0.026F^{0.56} \sinh^{-1}(17.6F^{0.0967}).$$

The predicted depths of the wave-mixed layer (h) calculated using eqs. 3 and 10 were compared with the depth of minimum sediment water content measured along each transect. Minima were only determined along transects where the sediment water content decreased below 75%, indicating some sediment erosion (Håkanson 1977). Other transects were excluded from the analysis. To test whether substrate morphology is related to sediment characteristics, the depth of the wave-mixed layer not accounted for by wave action (i.e., the difference between the observed depth of minimum water content and h, the predicted depth of the wave-mixed layer) was compared with substrate slope using multiple regression analysis on the following model:

$$(11) \quad R = a + b(S) + c(S \times F_{mean})$$

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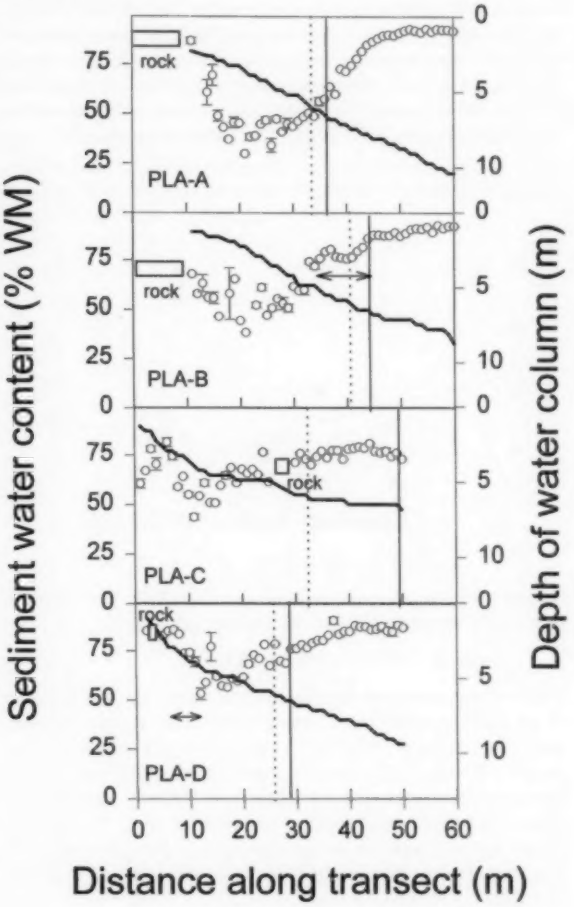
Fig. 4. Same as Fig. 2 in Plastic Lake. Boxes indicate rock substrates where cores could not be collected. Horizontal arrows indicate the extent of macrophyte beds (*Isoetes*). The metalimnion extends down to 12 m.



where R is the difference between the observed depth of the minimum sediment water content and the maximum depth of wave mixing predicted from eq. 10 (metres), S is slope (percent), and F_{mean} is mean fetch (metres) and a, b, and c are fitted coefficients. The interaction term ($S \times F_{mean}$) tests whether the effect of slope on R varies with site exposure.

The relationship between sediment organic and water content was tested using simple linear regression analysis on $\log_{10}$-transformed variables. Sediment organic matter contents in the shallow (<3 m) and deep (>6 m) portions of the littoral zone were compared using t-tests on arcsin-transformed variables (arcsin $\sqrt{OM}$, where OM is the proportion of organic matter in the sediments, grams per gram). All statistical analyses were performed in SAS for microcomputers, version 6.10.

Results

Patterns of sediment water content along depth gradients

The water content of littoral sediments ranged from 30 to >90% and generally showed a clear pattern of variation with depth (Figs. 2–4). Sediment water contents were generally high in shallow waters (<2–5 m), decreased sharply to 30–50% of fresh sediment mass further away from shore, and increased again in the deeper portion of the littoral zone (Figs. 2–4). Low sediment water contents (<75%) were measured at the end of two transects in Haynes and Paint lakes (HAY-D, PNT-B; Figs. 2 and 3), suggesting that these transects were too short to measure the depth of minimum sediment water content at these sites. The sediments along three transects showed no trend in sediment water content with increasing depth (HAY-A, HAY-C, PNT-C; Figs. 2–4). Sediment water contents remained high (>75%) along two transects in Haynes, the smallest lake (HAY-A, HAY-C; Fig. 2), and were lower (median = 67%) and more variable (range = 46–84%) along PNT-C (Fig. 3). The substrate along transect C in Paint Lake consisted of a patchwork of boulders and fine sediments, so it may not be surprising to observe large among-core variability along this transect. The distribution of sediment water content in the littoral zone varied among transects within a lake as well as among lakes.

The general patterns in sediment characteristics were, in some cases, altered slightly by the presence of thick macrophyte beds (horizontal arrows in Figs. 2–4). Sediments inside thick beds of *Myriophyllum* sp. (HAY-C, HAY-D) and *Isoetes* sp. (PLA-B) had lower water content than sediments at the edges of these beds (Figs. 2 and 4). No change in water content was observed at HAY-A, but the extent and the density of the *Myriophyllum* bed at this site are only approximate, since they were not recorded at the time of sampling. Very steep changes in sediment water content were observed in the thin mixed macrophyte bed at PNT-A (Fig. 3) and in the *Isoetes* bed at PLA-D (Fig. 4), but these changes were not unique to substrates covered by macrophytes (Figs. 2–4) and therefore cannot be attributed to the presence of macrophytes. The effect of macrophytes on sediment water content was small and was not consistent among sites.

Sediment characteristics in the shallow littoral zone

Sediments in the shallow portion of the littoral zone (<1.5 m) ranged from >80% water content to rocky shores where little or no sediments could be collected (Fig. 5). The most protected sites, with effective fetch <150 m, generally had very fluid sediments in the shallow littoral zone, suggesting low wave disturbance. Sediment water content decreased with increasing effective fetch up to 400–450 m where boulders were predominant and very little loose sediments were collected (Fig. 5).

Maximum depth of sediment mixing

Predictions of MDB depth using the model of Rowan et al. (1992) extrapolated well to small lakes with substrate slopes up to 27% (Fig. 6, points close to the 1:1 line). However, the model greatly overestimated the depth of the MDB along steeper slopes (two high points in Fig. 6).

I developed a similar model, based on measures of site exposure and substrate slope, to predict the maximum depth of sediment mixing in small lakes. Since low sediment water content is a good indication of sediment disturbance (Håkanson 1977), I used the depth of minimum sediment water content to estimate the maximum depth of sediment mixing in lakes. Minimum sediment water contents ranged between 1.4 and >7 m depths along different transects (Figs. 2–4). Low sediment water contents (<75%) were measured at the end of

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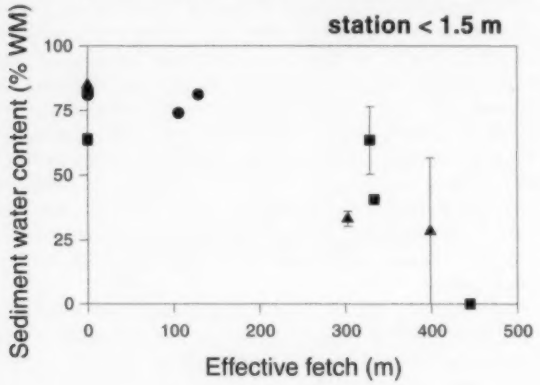
Fig. 5. Relationship between average sediment water content (WM, wet mass) in the shallow littoral zone (<1.5 m), including empty sediment cores, and effective fetch. Each point represents the average water content along the shallow portion of a transect. Transect PLA-A did not extend to depths <1.5 m and is not included in this graph. Error bars are among-core standard errors. Circles, Haynes Lake; squares, Paint Lake; triangles, Plastic Lake.



transects HAY-D and PNT-B (Figs. 2 and 3) and provide a minimum estimate of the depth of minimum water content at these sites (symbols with arrows in Fig. 7).

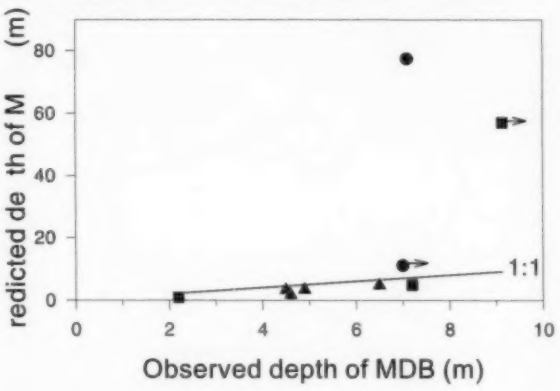
Minimum sediment water contents were found much deeper than the maximum wave-mixed layer (Fig. 7A). The size of waves and the depth of the wave-mixed layer were predicted from the exposure of each site (i.e., fetch) and very high wind speed (see Methods). Maximum wave-mixed layers ranged between 1 and 4 m, depending on the assumptions of the model, and increased with mean fetch of a site (lines in Fig. 7). Wave-mixed layers were slightly deeper when calculated from particle threshold velocities ($h_{U_{c3}}$) than when estimated as one half the wave length (h_{U2}; see Methods for details), and their depth increased with decreasing particle size and decreasing sediment density. The observed maximum depths of sediment mixing, estimated as the depth of minimum sediment water content, were as much as five times deeper than the maximum calculated depth of the wave-mixed layer (Fig. 7A).

The discrepancies between the observed maximum depth of sediment mixing and the depth of the wave-mixed layer were linearly related to the steepness of the substrate (Fig. 7B). Once the effect of wave action is taken into account (i.e., observed – $h_{U_{c3}}$), the depth of minimum sediment water content increases with increasing substrate slope ($r^2 = 0.82$, $P < 0.01$; Fig. 7B) but is not significantly related to the interaction term between slope and mean fetch (coefficient c in eq. 11, $P > 0.3$). Minimum sediment water contents on slopes steeper than 25% were found two to six times deeper than expected from wave action alone.

Sediment organic matter content

The organic matter content of littoral sediments was closely related to their water content (Fig. 8A), except along transect PNT-D where sediment organic matter content did not vary with depth and was uncorrelated with sediment water content

Fig. 6. Relationship between observed depth of the mud deposition boundaries (MDB, defined as 60% sediment water content, see Methods) in Haynes, Paint, and Plastic lakes and predictions from the model of Rowan et al. (1992). Each point represents one transect, with symbols as in Fig. 5. Symbols with arrows represent minimum estimates of the depth of MDB along transects where the lowest water content (<75%) was measured at the end of the transect; all other symbols represent measured MDB. Transects HAY-A, HAY-C, and PNT-C are not shown since sediment water contents remained >75%. The 1:1 line is added for reference.



(squares off the general relationship in Fig. 8A). The relationship can be described by the following equation:

$$\log(OM) = -4.7 + 3.0 \log(WC)$$

where OM is sediment organic matter content (as a percentage of dry mass) and WC is sediment water content (as a percentage of wet mass) ($r^2 = 0.77$, $P < 0.001$, $n = 519$, residual mean square = 0.028). The organic matter content of sediments with high water contents did not differ significantly between the upper (<3 m) and lower littoral zone (>6–8 m) in Haynes and Plastic lakes (t-tests on arcsin-transformed data, $P > 0.4$) and was only slightly higher in the lower littoral zone in Paint lake ($P < 0.001$; Fig. 8B).

Discussion

Sediment distribution in the littoral zone of small lakes

The sediments in stratified aquatic systems generally increase in water, organic, nutrient, and contaminant content with increasing depth (e.g., Sly 1978; Håkanson and Jansson 1983; Blais and Kalff 1995; Stephenson et al. 1995). Fine sediment particles at shallow sites are resuspended by waves and water currents (along with organic matter, nutrients, and contaminants) that transport them away from shore and "focus" them in deeper accumulation areas. Shallow sediments, therefore, are generally coarser and have lower water and organic contents than deep sediments.

Sediment distribution in small lakes differs from this general pattern. Sediment water and organic contents in Haynes, Paint, and Plastic lakes were often high in the upper littoral zone, decreased sharply between 1 and 7 m depth, and increased again in the lower littoral zone (Figs. 2–4). These differences in water content were measured in the surface 5 cm of sediments, and since sediment water content decreases with

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Fig. 7. (A) Relationship between maximum depth of sediment mixing (measured as depth of minimum sediment water content, see Methods) and mean fetch. Each symbol represents one transect. The curves indicate depths of the wave-mixed layers predicted from two models: the broken curve represents $h_{L/2}$ from eq. 3 and the solid curve is predicted from the particle threshold ($h_{U_{123}}$) using eq. 10. (B) Relationship between the residuals in Fig. 7A (i.e., observed depth of minimum sediment water content minus predicted depth of the wave-mixed layer, $h_{U_{123}}$, from eq. 10) and slope of the substrate (S). This relationship is described by $R = -1.0 + 0.15 \times S$ ($r^2 = 0.82$, $P < 0.01$, $n = 7$). Symbols as in Fig. 5. Symbols with arrows might underestimate the depth of minimum water content (HAY-D, PNT-B).

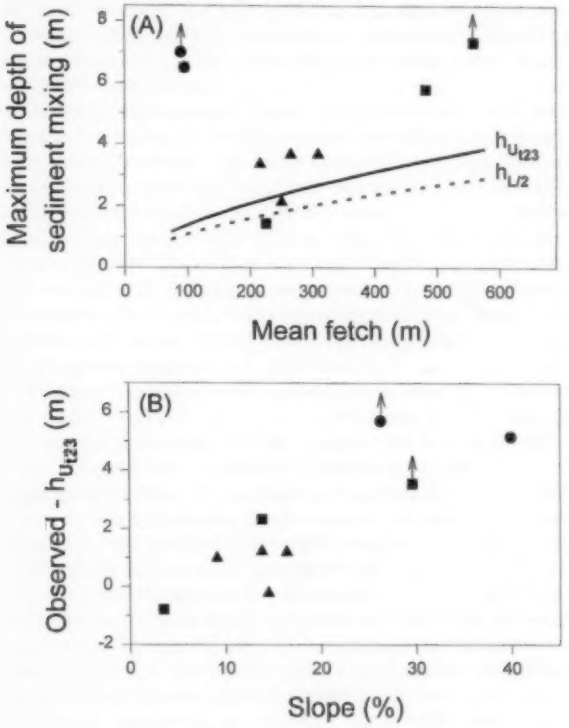
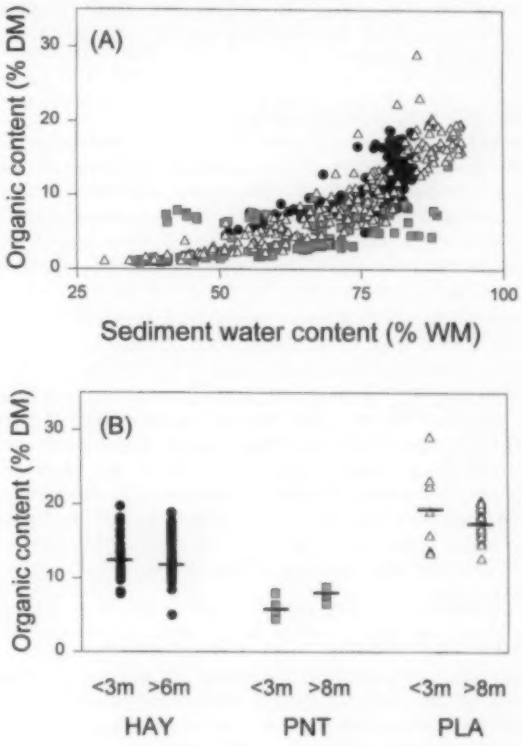


Fig. 8. (A) Relationship between sediment organic content (DM, dry mass) and sediment water content (WM, wet mass). Each point represents one core, with symbols as in Fig. 5. The equation describing this relationship is shown in the text. (B) Comparison of the organic content of sediments with high water content found in the upper (<3 m) and lower (>6 or 8 m) littoral zone. Each point represents one core, and the lines represent median sediment organic content. Sediment organic contents are not significantly different at different depths in Haynes and Plastic lakes (t-tests on arcsin-transformed data, $P > 0.4$), but are slightly higher in the lower littoral zone in Paint Lake ($P < 0.001$).



depth into the sediments (Håkanson and Jansson 1983), the differences may be even more pronounced in the surface 1–2 cm. Variations in sediment water content may be due to differences in particle size or differences in particle sorting. Preliminary data from transect PLA-B show little variation in mean particle size (mean equivalent spherical diameter by number = 10.4–11.2 μm) across a large gradient in sediment water content (45–95%; Fig. 4; H. Cyr, unpublished) and suggest that the gradient in sediment water content along this transect is due to differences in the intensity and (or) frequency of particle sorting.

The high water content of shallow sediments suggests that the upper littoral zone in small lakes is not necessarily a zone of erosion, but can be a transportation zone where fine particles settle temporarily (sensu Håkanson 1977). Within small lakes, average sediment water content at shallow sites increased with decreasing effective fetch (Fig. 5). Although the relationship may be weaker when exposed sites (effective fetch > 300 m), with and without sand particles, are included in the analysis, these results suggest that fine sediments are efficiently retained in the shallow littoral zone at the most protected sites (effective fetch < 150 m). The presence of fine sediments in the shallow littoral zone indicates low sediment disturbance and high retention of nutrients and organic matter, two factors that affect the abundance and productivity of benthic organisms.

Sharp declines in sediment water content were measured between 1 and 7 m along most transects (Figs. 2–4). Sediment water contents dropped to 30–50% over a few metres only, a sharp transition similar to that reported by Rowan et al. (1992) in larger lakes. The small scale of these patterns and their proximity to shore (within 20 m from shore) may partly explain why they have not been reported before. Most studies of sediment distribution have been done at much larger scales, with coarser resolution (minimum of 5–30 m between samples), and have focused on offshore sediments and accumulation zones rather than on the littoral zone (e.g., Håkanson and

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Jansson 1978; Rowan et al. 1992; Blais and Kalff 1995; Stephenson et al. 1995). The depth of minimum sediment water content is closely related to the maximal depth of the wave-mixed layer and to substrate slope (Fig. 7). This relationship suggests that sharp declines in sediment water content are remnants of past sediment mixing events.

In the lower littoral zone, sediment water content increased with depth (Figs. 2–4), a pattern similar to those reported in other aquatic systems (Sly 1978; Håkanson and Jansson 1983; Blais and Kalff 1995; Stephenson et al. 1995). Sediment water content increased to a plateau along several transects. These plateaus indicate areas of low sediment disturbance, but do not necessarily coincide with the long-term accumulation zones (Blais and Kalff 1995).

Sediments in macrophyte beds

Macrophyte beds reduce water flow and are well known to increase sedimentation of fine particles, especially at their edges (Petticrew and Kalff 1992; Losee and Wetzel 1993). Macrophyte beds had no discernible effect on steep changes in sediment water content along transects PNT-A and PLA-D (Figs. 3 and 4), but were associated with decreases in sediment water content at other sites (HAY-C, HAY-D, PLA-B). No clear drops in sediment water content were observed at HAY-A and HAY-B (Fig. 2), but the extent of the macrophyte beds at these sites is uncertain, since it was not recorded at the time of sampling. Small drops in sediment water content in the shallow portion of Haynes Lake were associated with thick water milfoil (*Myriophyllum*) beds (Fig. 2), and a similar pattern was observed along transect PLA-B in a thick bed of *Isoetes* (Fig. 4B). These drops in sediment water content were associated with drops in organic matter content in beds of *Myriophyllum*, but not in the *Isoetes* bed (data not shown). Macrophytes affect sediment water and organic matter content differently at different sites.

Effect of wave disturbance on sediment distribution

Waves are well known to resuspend sediments in shallow aquatic systems (e.g., Davis 1973; Håkanson 1977; Carper and Bachmann 1984; Demers et al. 1987). The depth of wave-induced sediment resuspension increases with increasing wind exposure and with decreasing size and density of sediment particles (Fig. 7A; Komar 1976). Calculations from hydrodynamic models suggest that direct wave action in small lakes can resuspend fine sediments down to a maximum depth of 1–4 m, depending on site exposure (lines in Fig. 7A). Smaller and lighter (e.g., more organic) sediment particles (i.e., $D <$ 23 μm, $\rho_s < 1.3$ g·cm^{-3}; see Methods) could be resuspended from slightly greater depths. Because these calculations are based on gale-force winds, which only occur a few times a year, they are likely to overestimate the depth of the wave-mixed layer during most of the summer. In small oligo-mesotrophic lakes, direct wave action affects only a small portion of the (euphotic) littoral zone during most of the summer.

As expected, sediment characteristics in the shallow, potentially wave-swept littoral zone (<1.5 m) varied systematically with effective fetch (Fig. 5). High average sediment water and organic contents were measured at very protected sites (effective fetch < 150 m) where the intensity of wave mixing was apparently insufficient to transport fine particles away. I noticed, however, that equipment left in the field for a few days became covered with fine sediments, which suggests that some sediment disturbance does occur, even at the most protected sites (e.g., HAY-A, HAY-C). More exposed sites (effective fetch > 300 m) had lower average sediment water contents (Fig. 5), characteristic of erosion zones (<50%; Håkanson 1977). These erosion zones had either relatively uniform sediments with low water content or very heterogeneous sediments with small pockets of fine sediments accumulating (temporarily) between boulders.

In stratified lakes, all sediments above the thermocline are potentially subject to wave disturbances. Sediments resuspended above the thermocline are mixed through the epilimnion and resediment during calm periods, in proportion to the depth of the water column (Hilton 1985). Complete mixing of epilimnetic sediments would result in (*i*) uniform sediment water content above the thermocline and (*ii*) increasing sediment organic content with increasing depth (Hilton 1985). Although erosion zones were clearly found at shallow exposed sites (<1.5 m, effective fetch > 150 m; Fig. 5), clear patterns in sediment water content were also observed within the epilimnion at PNT-A and at all sites in Plastic Lake (Figs. 3 and 4). Moreover, sediment organic content varied closely with sediment water content (Fig. 8A) and did not increase with increasing depth within the epilimnion. These observations suggest that sediment resuspension during summer in these small lakes is insufficient to homogenize the sediments throughout the exposed epilimnetic portion of the littoral zone. These results are consistent with low sediment resuspension measured in other stratified lakes during summer (e.g., Pennington 1974; Lastein 1976).

These results also suggest that in small clear lakes with relatively deep thermoclines, a portion of the warm littoral zone (i.e., above the thermocline) may be undisturbed during most of the summer. Because water temperature and physical disturbances both limit the biomass and productivity of organisms living in and on the sediments (Plante and Downing 1989; Lowe 1996; Cyr 1998), such warm low-disturbance areas are likely to be some of the most productive open-sediment (i.e., nonweeded) portions of the littoral zone. In small lakes (<500 ha), thermocline depth is positively related to water clarity (Fee et al. 1996). Small clear lakes tend to have deeper thermoclines than small eutrophic or dystrophic lakes and should have a larger portion of their warm littoral zone shielded from wave disturbances. Macrophyte growth (e.g., in meso-eutrophic lakes) alters significantly the extent of open-sediment areas and the biomass and composition of benthic communities (Cyr and Downing 1988).

Effect of slope on littoral sediments

Substrate morphology affects the stability of sediment particles and has been shown to influence sediment distribution in lakes. Rowan et al. (1992) modeled the effect of site exposure and of substrate slope on sediment distribution at a wide range of sites in 54 large lakes (area = 10 – 82 367 km^2). They found that the depth at which fine particles are deposited in lakes (i.e., MDBs) increases log-linearly with fetch (i.e., $\propto F^{0.74}$) and increases exponentially with substrate slope (i.e., $\propto 10^{\text{slope}}$). The model proposed by Rowan et al. (1992) extrapolates quite well to small lakes, but not to slopes >27% (Fig. 6). Data from Haynes, Paint, and Plastic lakes suggest that the maximum depth of sediment mixing (i.e., minimum sediment water

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content) is related linearly, not exponentially, to substrate slope (Fig. 7).

Rowan et al. (1992) reported that the maximum slope on which fine-grained sediments can be deposited varies between 7.5% in large lakes (maximum fetch > 50 km) and 15% in small lakes. Their data set, however, included only four sites representing small lakes (i.e., maximum fetch < 1 km). Håkanson (1977) found that no sediments were deposited on substrate steeper than 14% at deep sites (50–80 m) in Lake Vänern (surface area = 5650 km^2). In Haynes, Paint, and Plastic lakes, fine-grained sediments were often found on slopes steeper than 15% (HAY-A, HAY-B, HAY-C, HAY-D, PNT-B, PLA-A; Figs. 2–4), and the depth of minimum sediment water content, although strongly related to substrate slope, was usually located along a continuous, sometimes very steep slope. Sediment sliding and slumping requires more than a steep substrate.

Sediment movement along steep slopes was apparently not triggered by waves. I found no significant relationship between the extent of sediment sliding and slumping (i.e., observed – $h_{U_{93}}$ in Fig. 7B) and the interaction term between site exposure and substrate slope (coefficient c in eq. 11, $P > 0.3$). These results are consistent with the sharp declines in sediment water content observed by Håkanson (1977) between 50 and 80 m in Lake Vänern, far below the wave-mixed layer. My data and Håkanson's (1977) data suggest that substrate morphology can have an effect on sediment distribution, independent of the effect of wave action.

Several studies suggest that sediment resuspension is minimal during thermal stratification, but occurs mostly during lake turnover (Pennington 1974; Lastein 1976; Hilton 1985). Similarly, high sediment water contents were measured in large portions of the littoral zones of Haynes, Paint, and Plastic lakes during summer, but the distribution of sediment chlorophyll at different depths in these lakes suggests that the littoral sediments are thoroughly mixed during some period of the year (Cyr 1998). Sediment sliding and slumping, therefore, could be triggered by high water turbulence during periods of lake destratification in the fall and (or) spring. Further sampling at different times of the year will be necessary to test temporal patterns of sediment movements.

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Long-term changes in parasites of sockeye salmon (*Oncorhynchus nerka*) smolts

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Abstract: Parasites were examined in 41 annual samples of sockeye salmon (*Oncorhynchus nerka*) juveniles originating from lakes in British Columbia: Chilko and Shuswap. Nine species of parasitic helminths and two species of parasitic Crustacea were recovered from various organs. The number of species ranged from three to nine per year. Most (94%) parasites belonged to three tapeworm species, identified as core to the parasite community because they also infected the most hosts. Two of these cestode species are probably maintained by resident freshwater hosts in the lakes. Positive numerical associations were observed between four parasite species pairs. Parasite communities in both lakes were composed of similar species, with the exception of three rare Chilko Lake species that were never recovered from Shuswap Lake. Parasites were often as variable within stock (temporally) as between stocks (geographically), except for the prevalence of *Eubothrium* sp. and *Neoechinorhynchus* sp. and mean annual intensity of *Proteocephalus* sp. and encysted digenetic metacercariae, which were greater at Chilko Lake than at Shuswap Lake. Discrimination between the two lakes based on their parasite community is possible only in extreme cases of infection and thus is of limited use to fisheries officers attempting to distinguish between these stocks.

Résumé : Nous avons étudié les parasites de 41 échantillons annuels de saumon rouge (*Oncorhyncus nerka*) juvénile provenant des lacs Chilko et Shuswap, en Colombie-Britannique : nous avons trouvé neuf espèces d'helminthes et deux de crustacés dans divers organes et dénombré, selon l'année, de trois à neuf espèces. La plupart (94%) des parasites appartenaient à l'une ou l'autre de trois espèces de cestodes, celles-ci formant pour ainsi dire le coeur de la communauté des parasites, car elles infectaient aussi le plus grand nombre d'hôtes. Deux de ces cestodes se maintiennent probablement grâce à des espèces hôtes dulcicoles résidentes des lacs. Nous avons mis en évidence des associations numériques positives entre quatre paires d'espèces de parasites. Les communautés de parasites des deux lacs avaient une composition spécifique semblable, sauf pour trois espèces rares dans le lac Chilko et jamais trouvées dans le lac Shuswap. Le parasitisme était souvent aussi variable dans un même stock (variation temporelle) que d'un stock à l'autre (variation géographique); il y a toutefois des exceptions : ainsi, la prévalence d'*Eubothrium* sp. et de *Neoechinorhynchus* sp. et de même que la fréquence annuelle moyenne de *Proteocephalus* sp. et des métacercaires étaient plus élevées dans le lac Chilko que dans le lac Shuswap. Comme il n'est pas possible de distinguer les deux lacs l'un de l'autre d'après leurs communautés de parasites respectives, sauf en cas d'infection extrême, ce paramètre n'est que d'une utilité limitée aux gestionnaires des pêches qui cherchent à différencier ces stocks. [Traduit par la Rédaction]

Introduction

Sockeye salmon (*Oncorhynchus nerka*) are an economically important food fish that spend their first year of life in nursery lakes. Many studies have examined the potential of parasites as biological tags to indicate the freshwater lake origin of oceanic salmon populations (Margolis 1965; Bailey and Margolis 1987; Bailey et al. 1988, 1989). Parasites most suitable as biological tags for anadromous salmon stocks are those that infect young salmon in the home system, differ in abundance among home systems, and remain with the fish throughout the time of study (Bailey et al. 1989). Moser (1991) added that they should be easily detectable and not cause large-scale selective mortality or behavioural changes in the host. One difficulty in establishing parasites as biological tags is that differences in parasite prevalence and intensity within a stock over time may be greater than differences between stocks. Bailey and Margolis (1987) described some 20 species of parasites in the taxa Protozoa, Monogenea, Digenea, Cestoda, Nematoda, Acanthocephala, and Crustacea in sockeye smolts from British Columbia lakes. They noted significant interannual variation in five of eight parasites in 3 years of samples from Chilko Lake. Long-term trends can compromise the value of a parasite to serve as a biological tag. Vancouver Island stocks that were once differentiated by the presence or absence of *Myxobilus arcticus* and *Henneguya* sp. have become indistinguishable as infections gradually declined (T. McDonald, Pacific Biological Station, 3190 Hammond Bay Road, Nanaimo, BC V9R 5K6, Canada, personal communication). Moser (1991) took into account considerable interannual variation over a 10-year study of Pacific herring (*Clupea pallasi*) parasites in his selection of tags.

The International Pacific Salmon Fisheries Commission and Canadian Department of Fisheries and Oceans collected 40 years worth of sockeye smolt samples from Chilko Lake of the Fraser River drainage basin. This offers the unique

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opportunity to test the compound hypothesis that parasite communities vary significantly over time and, by comparing a long-term parasite sample from Chilko Lake with that of another lake in the same drainage basin (Shuswap Lake), that parasites vary more within stock between years than between stocks. To this end, we examine quantitative and qualitative stability in the parasite community of Chilko Lake and to a lesser extent Shuswap Lake. We test for significant quantitative annual variation in parasite prevalence and mean intensity. We examine qualitative parasite community characteristics such as species composition, relative abundances of parasite species, species relationships, and parasite species diversity, asking how these attributes vary annually. Finally, we examine the discriminant ability of parasite communities to determine lake of origin between Chilko Lake and Shuswap Lake stocks. Because environment is an important determinant of parasite communities (Bailey and Margolis 1987), we predict that parasite communities of sockeye from the same drainage basin will be similar in species composition. That said, we also predict that because sockeye are known to vary in abundance from year to year, their parasites will also be expected to do so. Such variation could limit the use of parasites as biological tags.

Materials and methods

Fresh-frozen (sample year 1992) and fixed (all other sample years) sockeye smolts from Chilko Lake (51°16′N, 124°04′W) and Shuswap Lake (50°56′N, 119°17′W), from the Fraser River drainage basin, British Columbia, were examined for parasites. Sockeye have on average a 4-year life cycle beginning when eggs are deposited in gravel river/lake beds during autumn spawning. Emergence occurs in spring. Juvenile salmon undergo a growth period of 1 year on average in freshwater. This stage is referred to as fry. At the end of the freshwater period, juvenile salmon undergo physiological changes to prepare for seaward migration and marine survival. This stage is referred to as smolt. Sampling occurred annually during spring seaward migration, using fence and dip net. Field sampling spanned the month-long migration period, collecting salmon smolts. Daily samples were usually pooled in the field, resulting in a single sample of smolts per year. Exceptions are those years in which samples were taken twice a year, resulting in the collection of fry during fall sampling and smolts during spring sampling. Those years were 1991 samples from Chilko Lake and 1989 and 1990 samples from Shuswap Lake. In 1992, Chilko Lake was sampled daily throughout the migration period at the request of the researchers. We wanted to ensure that timing of migration was independent of parasite infection, thus ruling out differences in sampling date as a source of variation.

Field teams usually terminated smolts by freezing and transported them to DFO's Cultus Lake storage facilities. Here, they were transferred to 10% formalin and stored. The 1992 Chilko Lake samples were sent directly to Vancouver, frozen. We subsampled at least 25 smolts per year from 37 Chilko Lake annual samples from 1950 to 1992, except 1967 ($n = 10$), 1970 ($n = 24$), and 1990 ($n = 10$), when smolt numbers were low, and omitting 1951, 1957–1958, and 1964 for which smolts had not been sampled. Shuswap Lake was also included for comparison, but smolt samples existed for only a few years: necropsies were performed on 25 smolts per year from 1989 to 1991 and 1993. Prior to examination, formalin-fixed samples were rinsed and soaked in tap water for 24 h. This procedure was repeated two more times before specimens were transferred to 70% ethanol.

Parasite collection

We carried out detailed dissections of sockeye smolts, systematically searching every major organ for macroparasites. Microparasites were not considered because the method of host preservation makes them difficult to retrieve. After wet-weighing and measuring (fork length) a fish, we examined its external surface, gill opercula, gills, eyes (interior and exterior), body cavity, liver, heart, digestive tract (interior and exterior), and gonads using a dissecting microscope and alternating light sources (transmitted and incident light). Parasites were typed and enumerated. Parasite types were identified to species whenever possible. Voucher specimens of each parasite were saved regularly and stored in 70% ethanol. This facilitated the training of data collectors and ensured quick and accurate identification of parasites. Data collection was overseen by one of the researchers (S.N.B.) to ensure continuity.

Data analysis

Parasites are summarized in terms of prevalence, mean intensity, and abundance. Prevalence is the percentage of fish in a sample infected with a given species of parasite, mean intensity is the mean number of parasites of a given species per infected fish in a sample, and abundance is the number of parasites in a sample divided by the number of fish sampled (Margolis et al. 1982). None of these variables turned out to be normal or Poisson distributed. Appropriate transformations (Zar 1996) were made for all analyses unless otherwise stated. Prevalences were broken down into counts of infected fish and respective sample sizes, n, and then recalculated with the transformation $0.5 \cdot \arcsin(\text{count}/(n + 1))^{1/2} + 0.5 \cdot \arcsin((\text{count} + 1)/(n + 1))^{1/2}$. Intensities, mean intensities, and abundances were transformed by $\ln(\text{variable} + 1)$.

The results of this study depend on whether we adequately sampled the parasite community. Hence, we wanted to establish whether 25 fish were sufficient to detect most of the parasite species in a year's parasite community. Species accumulation curves for each year displayed the total number of parasite species found within a year-class and the sample size in which that number was first reached. These were fit with an exponential model of species accumulation, which assumes that the probability of discovering a new species decreases in proportion to the current size of the species list (Walther et. al. 1995). The true species richness was thus estimated ("true" being the hypothetical number of species recovered if sample size was equivalent to population size), as well as the sample size in which 95% of these species would be detected. In this way, we can investigate sampling effort as a source of annual variation.

To address the issue of stability in parasites over time, we took a quantitative and qualitative approach. Quantitative stability would be indicated by low annual variation in parasite abundances, prevalences, and mean intensities. Annual variation in each parasite's abundance, intensity, prevalence, and species diversity index was examined using Kruskal–Wallace rank sum analysis of variance. Annual variation in mean intensity and prevalence was quantified for each parasite species relative to overall mean intensity and mean prevalence using coefficients of variation (CV = (standard deviation/mean)·100% (Zar 1996)). Qualitative stability would be indicated by the consistent presence of the same species in the community from year to year and by the maintenance of their relative abundances across years, as examined below.

Species relationships

Community structure was examined with respect to relative abundance of species present, classifying species as "core," "secondary," and "satellite" for common to rare species. Method of classification was set out by Bush and Holmes (1986). These terms are loosely based on theoretical work on the frequency of species (Hanski 1982 for review) and emphasize each parasite's relative importance in the parasite community and presumably to the host.

Correlation between species can indicate competition if negative or shared life cycle traits if positive. Parasite species interactions were examined in three ways: (*i*) we calculated Spearman's rank correlations between species pairs using three statistical summaries of yearly

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data: prevalence, mean intensity, and abundance, (*ii*) nonrandom species assortment was assessed within each year-class using contingency tables and Fisher's exact test for nonrandomness to detect transient annual associations that could reflect changes in lake environments such as intermediate host population levels, and (*iii*) samples were combined over all years and assessed for nonrandom species assortment using chi-square goodness-of-fit to random expected frequencies in presence–absence contingency tables. Significance (alpha) for each test was set at 0.05 divided by the test's rank according to p-value from smallest to largest (sequential Bonferroni adjustment, Rice 1989). Analyses were performed in S Plus, a programming environment for graphics and data analysis (Becker et al. 1988).

Diversity

Diversity of the parasite community was examined in two ways: (*i*) a mean diversity index and evenness index were calculated for each year-class by averaging Brillouin's indices for a fully censused community (Magurran 1988) over all fish sampled in the year-class and (*ii*) fish from a year-class were pooled to calculate an annual diversity index, being the inverse Simpson's index (Kemp's index) chosen for its insensitivity to changes in sample size (Magurran 1988). Parasite diversity indices for each fish's parasite community (Brillouin's index) and the cohort's parasite community (Kemp's index) are used to examine systematic changes in annual diversity and evenness. Samples taken of fry and smolts from the same year-class (Chilko Lake in 1991 and Shuswap Lake in 1989 and 1990) had equal variances (nonparametric version of Bartlett's, $p < 0.05$) and similar parasite communities (by-species comparison, Wilcoxon rank sum test) and so were pooled. The 1992 Chilko Lake samples spanning the month-long migration period were also pooled, as there were no differences in parasite community throughout the sampling period (Spearman's rank correlation, species abundance by date).

Ranked parasite species abundance plots (relative abundance of each parasite species against its corresponding rank according to that abundance) were developed and tested against several theoretical models (geometric, log series, lognormal, broken stick) using log-likelihood tests (alpha < 0.05) (Magurran 1988). This renders it comparable with other communities of species and indicates the diversity and general colonization pattern of the system.

Variability within the Chilko Lake parasite community relative to that of Shuswap Lake

Parasite mean intensity and prevalence were tested for differences between Chilko and Shuswap lakes to detect whether the variation within a lake was large enough to mask differences between lakes. Multiple tests were corrected for by the Bonferroni sequential adjustment (Rice 1989).

In a final comparison of the two parasite communities, we attempted to discriminate between stocks based on the 11 parasite species found in this study. Discriminant function analysis, Fisher's linear method, generated functions of parasite variables that maximized the difference between lakes. The null hypothesis that these discriminant variable means are the same for each lake was tested with an F-test on Z-transformed Mahalanobis' generalized distances. The ability of the discriminant function to correctly assign fish to either lake was assessed by reclassifying the data according to the discriminant rule and calculating the reclassification error rate. Parasite variable contribution to the discriminant function was also considered (Dillon and Goldstein 1984). Discriminant function analysis will have elevated misclassification rates if the assumptions of identical variance–covariance matrices between lakes and multivariate normality are violated (Dillon and Goldstein 1984). In an attempt equalize variance and approach a multivariate normal distribution, parasite counts were ln(counts + 1) transformed after removing fish that were completely uninfected.

Results

Parasite community

Eleven species of parasites were found in sockeye smolts from Chilko and Shuswap lakes. They include the cestodes *Proteocephalus* sp. (immature), *Eubothrium* sp. (immature), and *Diphyllobothrium ditremum* (plerocercoid), acanthocephalans of the genus *Neoechinorhynchus*, the digeneans *Tetracotyle* sp. (metacercaria), *Diplostomum* sp. (metacercaria), *Crepidostomum farionis* (Chilko Lake only), as well as encysted unidentified digenetic metacercariae of a single type presumed to be of the same species, the nematode *Philonema agubernaculum*, and copepods *Salmincola californiensis* (Chilko Lake only) and *Ergasilus nerka* (Chilko Lake only). Acanthocephalans recovered were all juveniles of one of two species: *Neoechinorhynchus rutili* or *Neoechinorhynchus salmonis* (see Ching 1984). Lack of distinguishing adult characteristics and poor quality of preservation did not allow us to assign acanthocephalans to a particular species. *Philonema* sp. individuals were also juveniles and hence were difficult to assign to a species. *Philonema agubernaculum* is the most probable based on previous findings (Bailey and Margolis 1987). Sites of infection of the parasites recovered in this study are identical to those reported by Bailey and Margolis (1987, table 2). Of the parasites recovered in this study, *Proteocephalus* sp. occurred at the highest intensity, although *Diphyllobothrium ditremum* was the most prevalent species. *Eubothrium* sp. was the third most abundant overall. The least prevalent parasites at Chilko Lake were *Crepidostomum farionis*, *Ergasilus nerka*, *Salmincola californiensis*, and *Philonema agubernaculum*, the latter three of which had in addition only one individual per infection. *Philonema agubernaculum* was much more common at Shuswap Lake.

Parasite infections were independent of migration timing: 1992 Chilko Lake samples, where fish were taken daily throughout the migration period, did not vary in parasite intensity or prevalence with date. Results were not artifacts of sample size: in 34 of 37 years of Chilko Lake samples, the rate of parasite species accumulation plateaued, indicating that sample size was sufficient to detect 95% of the parasites present. Exceptions are 1966, 1989, and 1991, which accumulated species linearly rather than logarithmically. These years consist of many species that are equally rare (high evenness).

Prevalence, mean intensity, and abundance for all but the rarest parasite species varied significantly from year to year (Kruskal–Wallace rank sum ANOVA; $p < 0.001$) but with no consistent pattern over the 40-year study period (Fig. 1). Thus, there is a lack of quantitative stability or consistency to the parasites of Chilko Lake smolts, although some parasites vary in prevalence and mean intensity less than others. *Proteocephalus* sp., *Eubothrium* sp., *Diphyllobothrium ditremum*, and *Tetracotyle* sp. were least variable in abundance, evidenced by the low CV for both mean prevalence and overall mean intensity (Table 1).

Species relationships

Core species *Proteocephalus* sp., *Eubothrium* sp., and *Diphyllobothrium ditremum* collectively accounted for 94% of all worms (Fig. 2; Table 1). These also dominated fish parasite communities by having the highest numbers of individuals per

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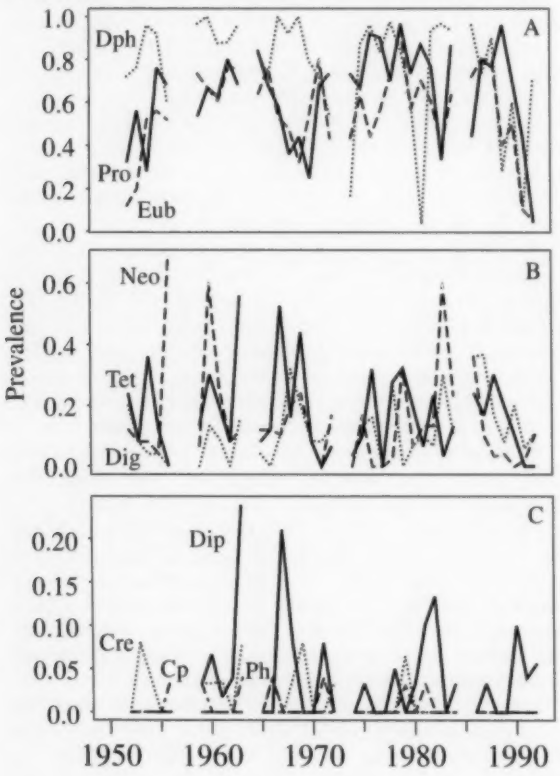
Fig. 1. Prevalence of parasite species in sockeye salmon smolts from Chilko Lake from 1950 to 1992. Lines of prevalence are broken where there are missing years in the data: 1951, 1957, 1958, 1964, 1973, and 1985. Parasite species are grouped into three subplots based on how common they are: (A) most common parasites: cestodes *Proteocephalus* sp. (Pro, solid line), *Eubothrium* sp. (Eub, broken line), and *Diphyllobothrium ditremum* (Dph, dotted line); (B) moderately common parasites: digenean *Tetracotyle* sp. (Tet, solid line), acanthocephalan *Neoechinorhynchus* sp. (Neo, broken line), and unidentified digenetic metacercariae (Dig, dotted line); (C) rarest parasites: digeneans *Diplostomum* sp. (Dip, solid line) and *Crepidostomum farionis* (Cre, dotted line), copepods *Ergasilus nerka* and *Salmincola californiensis* (pooled) (Cp, broken dotted line between 1955 and 1960; at other times, it is zero and overlaps with other lines), and nematode *Philonema agubernaculum* (Ph, broken line).



fish. Secondary species were *Tetracotyle* sp., *Neoechinorhynchus* sp., and digenetic metacercariae, representing collectively 5.6% of parasites found and infecting 12–15% of the hosts. Satellite species include *Crepidostomum farionis*, *Diplostomum* sp., *Philonema agubernaculum*, *Salmincola californiensis*, and *Ergasilus nerka* (collectively 0.12% of parasites and up to 4% of the hosts) (Table 1).

In most years, core species retained their numerical dominance over the rest of the parasite community in spite of annual variation in numbers. *Proteocephalus* sp., *Eubothrium* sp., and *Diphyllobothrium ditremum* were the most prevalent species in 28 of 37 years sampled. Exceptions were years in which secondary species were more prevalent than core because (*i*) one or more core species dropped 2 standard deviations below average prevalence (1952, 1981, and 1992) or (*ii*) secondary species were simply very prevalent (1954, 1956, 1969, and 1983). In 1952, *Eubothrium* sp. prevalence dropped to 12%, behind that of *Tetracotyle* sp. at 24%. In 1981, *Diphyllobothrium ditremum* prevalence was 3%, lower than most other species that year. In 1992, prevalence of *Proteocephalus* sp. and *Eubothrium* sp. (4 and 5%, respectively) was lower than that of all other species present that year. In 1954, prevalence of secondary species *Tetracotyle* sp. was 36%, greater than that of *Proteocephalus* sp. at 28%. In 1956, prevalence of *Neoechinorhynchus* sp. was 68%, 2 standard deviations above its overall mean and greater than that of both *Eubothrium* sp. and *Diphyllobothrium ditremum* (52 and 60%, respectively). In 1969, prevalence of *Tetracotyle* sp. was 44%, greater than that of *Eubothrium* sp. at 32%. In 1983, prevalence of *Neoechinorhynchus* sp. was 60%, greater than that of *Proteocephalus* sp. and *Eubothrium* sp. (35 and 40%, respectively). *Proteocephalus* sp., *Eubothrium* sp., and *Diphyllobothrium ditremum* were the only parasites consistently recovered every year from the Chilko Lake community. *Tetracotyle* sp. and *Neoechinorhynchus* sp. were recovered most years. All others were present no more than half the years sampled. Thus, there is a qualitative stability to the parasite community because certain core parasites are consistently present, and parasites usually maintain the same relative abundances.

Species interactions were investigated in three ways: Spearman's rank correlations between species, contingency tables of each species pairs' presence–absence for each year separately, and contingency tables of each species pairs' presence–absence for all years combined. No statistically significant species interactions were found by contingency table presence–absence for any of the years analyzed separately. However, *Diphyllobothrium ditremum* and *Tetracotyle* sp. are positively associated according to both Spearman's rank correlations on prevalence and mean abundance (rho = 0.6, $p = 0.0003$ and rho = 0.532, $p = 0.0014$, respectively) and a contingency table for presence–absence done over all years combined ($p = 0.0002$). Strong correlations were also observed between *Proteocephalus* sp. and *Eubothrium* sp. prevalences ($r = 0.552$, $p = 0.0004$) and between *Eubothrium* sp. and *Diphyllobothrium ditremum* mean intensities (rho = 0.519, $p = 0.0018$). The former relationship proved to be significant according to Pearson's product moment test for correlation, which was deemed appropriate after the variables had been transformed. These positive tapeworm associations were supported by the results of contingency tables for presence–absence done over all years combined (*Proteocephalus* sp. and *Eubothrium* sp., $p < 0.001$; *Eubothrium* sp. and *Diphyllobothrium ditremum*, $p < 0.001$). Other species pairs showed positive associations in contingency tables for presence–absence done over all years combined (*Eubothrium* sp. and *Tetracotyle* sp., $p < 0.001$; *Neoechinorhynchus* sp. and *Diphyllobothrium ditremum*, $p < 0.001$), but these relationships were unsupported by the other two methods of analysis.

Diversity

Annual species richness varied from 3 in 1977 to 9 in 1963. This variation is independent of sample size and does not show any pattern over time (Fig. 3). The overall species richness at

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Table 1. Summary of parasite infection and variability in sockeye salmon smolts from Chilko Lake.

Parasite species	Overall mean prevalence[a]	CV (%)[b]	Overall mean intensity[a]	CV (%)[b]	% of years present	% of total parasites
Core						
Proteocephalus sp. immature	0.64	33.81	23.86	56.95	100.0	
Diphyllobothrium ditremum plerocercoid	0.75	36.50	3.49	62.40	100.0	94
Eubothrium sp. immature	0.55	38.33	2.83	52.99	100.0	
Secondary						
Digenetic metacercaria	0.12	85.98	3.06	88.72	81.1	
Tetracotyle sp. metacercaria	0.17	82.89	1.35	54.47	86.5	5.6
Neoechinorhynchus sp.	0.15	112.96	2.13	126.76	86.5	
Satellite						
Diplostomulum sp. metacercaria	0.04	149.46	1.07	100.81	51.35	
Crepidostomum farionis	0.02	152.76	1.54	165.15	35.14	
Philonema sp.	0.01	231.57	1.00	230.44	16.22	0.12
Salmincola californiensis	—[c]	608.28	1.00	608.28	2.70	
Ergasilus nerka	—[c]	608.28	1.00	608.28	2.70	

Note: Species are grouped into three categories separated based on their relative abundance from highest percentage of parasites to lowest: "core," "secondary," and "satellite."

[a] $n = 37$ years.

[b] CV = (SD/mean)·100.

[c] Occurred only once over all years: prevalence approaches zero.

Chilko Lake was 10 species of parasites and at Shuswap Lake was 7. Diversity and evenness of the parasite community, represented by Brillouin's and Kemp's indices, varied significantly from year to year (Kruskal–Wallace rank sum ANOVA; $p < 0.001$). However, when Kemp's index was examined for autocorrelation over time, up to 4 years ahead (duration of the sockeye life cycle), no temporal pattern was detected in this variation. Nor was it correlated with parasite abundance, host size, or host population size.

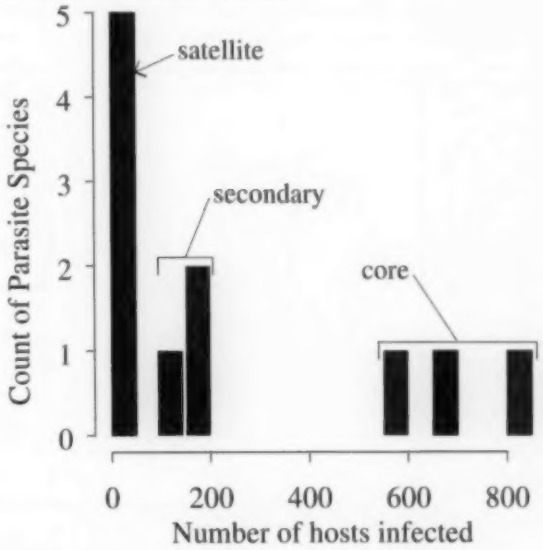
The rank abundance plot (abundance of individuals in each species, ranked by their abundances) for all years pooled is best described by log series or lognormal distribution (log-likelihood ratio goodness-of-fit test; $p > 0.75$ and $p > 0.5$, respectively). Rank species abundance distributions of individual years varied between a log series and a lognormal fit (Fig. 4). Years 1953 and 1992 had the most even distribution of species across hosts; their curves were the flattest.

Variability within the Chilko Lake parasite community relative to that of Shuswap Lake

The parasite community in Shuswap Lake smolts is similar in composition to that of Chilko Lake smolts. *Proteocephalus* sp., *Eubothrium* sp., and *Diphyllobothrium ditremum* were most prevalent and occurred at highest intensity in Shuswap Lake. However, prevalence and intensity of all species were generally lower at Shuswap Lake than at Chilko Lake: in some cases, these differences are significant (Table 2). *Crepidostomum farionis*, *Ergasilus nerka*, and *Salmincola californiensis*, all rare species in Chilko Lake, were missing from Shuswap Lake.

Discriminant function analysis yielded an 11 (the number of parasite species) by 11 matrix corresponding to 11 discriminant functions each with 11 weights, one for each parasite species, and denoted b (available from corresponding author). The first two discriminant functions were matrix-multiplied with the parasite data from each lake, thus projecting the two lakes onto a subspace designed to maximize their differences

Fig. 2. Frequency histogram of number of parasite species recovered from sockeye salmon smolts from Chilko Lake between 1950 and 1992, falling into abundance classes identifying "core," "secondary," and "satellite" parasites.



(Fig. 5). Overlap between lakes is extensive. However, the means of the two lakes' parasites were found to be significantly different ($Z_{0.01;11,1143} = 11.79$, $p < 0.001$). The discriminant rule derived from the first discriminant function is as follows: if fish $x < 0.238$, classify sample as coming from Chilko Lake, and if fish $x > 0.238$, classify sample as coming from Shuswap Lake, where

$$\text{Fish } x = \sum_{i=1}^{11} b_{i,\text{DV1}} \cdot \text{fish parasite}_i.$$

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Fig. 3. (A) Parasite species richness in sockeye salmon smolts from Chilko Lake between 1950 and 1992 examined as a function of sampling effort (sample size). Points are labeled by year. r is Spearman's rank correlation coefficient and p is the probability that this deviates from zero by chance alone. (B) Parasite species richness in sockeye salmon smolts from Chilko Lake between 1950 and 1992 examined with time in years.

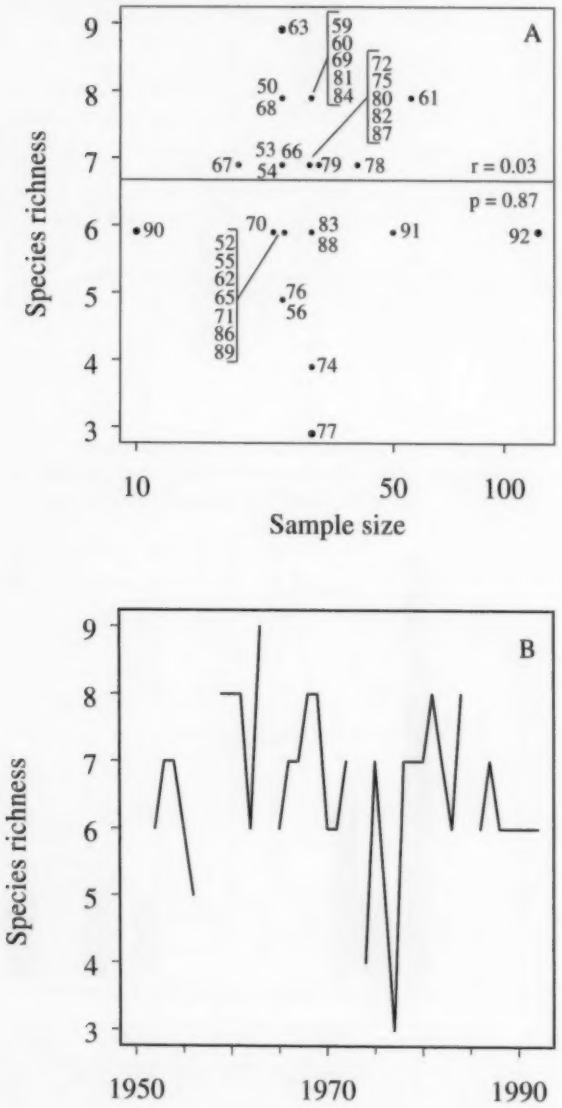
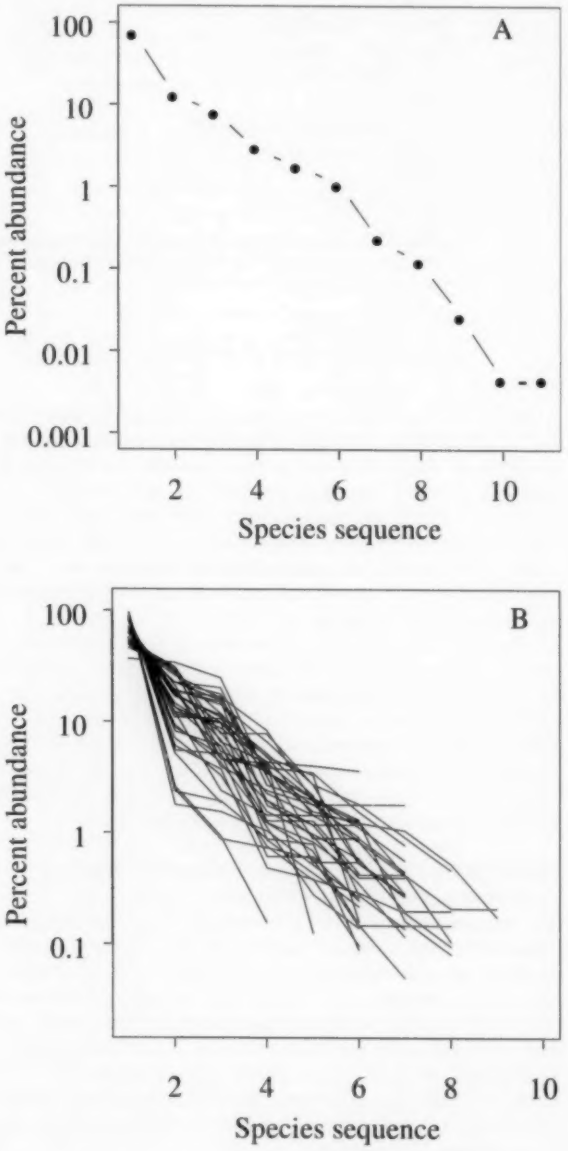


Fig. 4. Parasite species from sockeye salmon smolts from Chilko Lake between 1950 and 1992 ranked according to abundance. (A) Ranked parasite species plotted against their relative abundance, calculated over all years. The curve best fits both lognormal and log series species distribution models. (B) Ranked parasite species plotted against their relative abundance for each year individually. Years vary in fit between a lognormal and a log series model for individuals' distribution across species.



The critical point for classification is marked by a vertical line on Fig. 5. Using this procedure on the original data, the discriminant rule correctly reclassified 92.47% of the fish. However, misclassification rates differed greatly between the individual lakes: Chilko Lake fish were correctly reclassified 99.43% of the time whereas Shuswap Lake fish were correctly reclassified only 13.83% of the time. Those fish most likely to be misclassified form a group immediately left of the critical point and have low to absent infections for each parasite. The center (Fig. 5, between 2 and 6 on the first discriminant axis) mixed-lake group's fish were all infected with a single nematode. By contrast, fish that stand out as unique to Shuswap Lake (upper right area of Fig. 5, greater than 6 on the first discriminant axis) are those with multiple infections of

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Table 2. Comparison of Chilko and Shuswap lakes sockeye salmon smolt parasite infections.

	Mean prevalence			Mean intensity		
Parasite species	Chilko Lake	Shuswap Lake	*p*-value	Chilko Lake	Shuswap Lake	*p*-value
Eubothrium sp. immature	0.55	0.12	0.0002[a]	2.83	1.36	0.0226
Neoechinorhynchus sp.	0.15	0.02	0.0029[a]	2.13	2.33	0.0902
Proteocephalus sp. immature	0.64	0.34	0.0154	23.86	6.03	0[a]
Unidentified digenetic metacercaria	0.12	0.02	0.0486	3.06	1.00	0.0012[a]
Tetracotyle sp.	0.17	0.05	0.0524	1.35	1.90	0.2132
Diphyllobothrium ditremum plerocercoid	0.75	0.46	0.0586	3.49	2.06	0.1461
Philonema agubernaculum	0.01	0.12	0.2617	1.00	1.23	0.6725
Diplostomulum sp.	0.04	0.02	0.2954	1.07	1	0.1567
Salmincola californiensis	—[b]	0		1	na	
Ergasilus nerka	—[b]	0		1	na	
Crepidostomum farionis	0.02	0		1.54	na	

Note: The *p*-values represent the two-tailed probability that Shuswap Lake differs from Chilko Lake (Student's *t*-test or Welch *t* if sample variances are unequal).

[a]Significant at the Bonferroni-corrected alpha level appropriate for the test order.

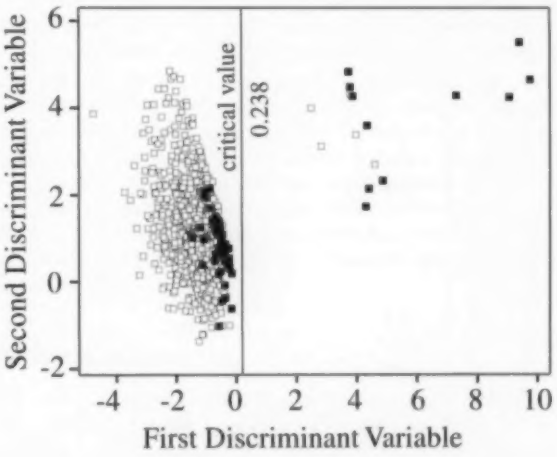
[b]Occurred only once over all years: mean prevalence approaches zero.

Philonema agubernaculum. Those that stand out as unique to Chilko Lake (extreme left side of Fig. 5, less than –2 on the first discriminant axis) have heavy infections of at least one of the tapeworms, inhabiting the upper left corner of Fig. 5 if they are also infected with digenetic metacercariae or the lower left corner if they are not. The importance of these parasite species in discrimination can be rated by standardizing the first discriminant variable for intercorrelations and ranking the subsequent weights: *Philonema agubernaculum* is ranked first, followed by the three tapeworms, ranked 2–4.

Discussion

The parasite community of Chilko Lake is largely dependent on other hosts in the lake, since many of the parasites recovered are presumably not maintained by sockeye. Leong and Holmes (1981) noted that parasite exchange often occurs between hosts that are taxonomically related and (or) abundant. Two of the three core species are maintained by other lake-resident fish. Mature *Proteocephalus* sp., which gave rise to the most intense infections, does not occur in sockeye — the presence of juvenile forms in smolts is temporary (Bailey et al. 1989). Lake-resident fish such as threespine stickleback (*Gasterosteus aculeatus*), rainbow trout (*Oncorhynchus mykiss*), mountain whitefish (*Prosopium williamsoni*), lake whitefish (*Coregonus clupeaformis*), and Dolly Varden (*Salvelinus malma*) maintain the infection by acting as final hosts (host distribution: Chilko Lake Study Team 1993; parasite distribution: McDonald and Margolis 1995). *Eubothrium* sp., the third most abundant parasite, is similarly freshwater (Bailey et al. 1989). In some lakes (e.g., Cultus Lake and Babine Lake), *Eubothrium* sp. mature in sockeye smolts within the year-long lake-resident stage (Boyce 1974). However, all forms recovered in this study were immature: sockeye are presumably a dead end for these parasites, for which lake-resident fish such as rainbow trout, chinook salmon (*Oncorhynchus tshawytscha*), Dolly Varden, coho salmon (*Oncorhynchus kisutch*), and mountain whitefish presumably act as normal final hosts (host distribution: Chilko Lake Study Team 1993; parasite distribution: McDonald and Margolis 1995).

Fig. 5. Results of a linear discriminant function analysis to segregate Chilko and Shuswap lakes based on their sockeye salmon smolt parasites, sampled annually for 38 years between 1950 and 1992. Shuswap Lake fish are represented by solid squares, and Chilko Lake fish are represented by open squares. The first and second discriminant variables out of a possible 11 are plotted. The vertical line represents the critical value of the discriminant rule: if a fish lies to the right, it is classified as originating from Shuswap Lake; if it lies to the left, it is classified as originating from Chilko Lake.



Of the few long-term parasitological studies that exist, each points to different factors affecting parasitic infections over time. Factors that give rise to declining trends in parasitic infections are competition between parasites following an introduction (Kennedy 1985), decreasing intermediate host population levels (Smith 1973), and environmental factors such as lake eutrophication (Esch et al. 1986) or human management (Kennedy 1993). Kennedy (1985) observed no trend in *Tylodelphys* sp. infections of perch: instead, parasites showed oscillations in abundance unrelated to fish host

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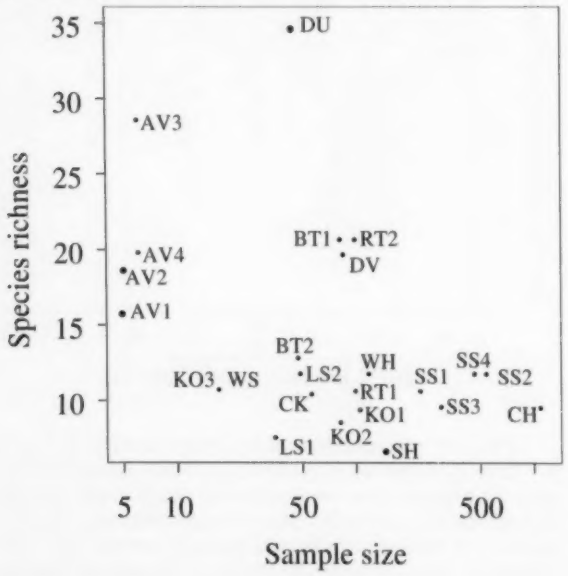
Fig. 6. Comparison of macroparasite species richness in sockeye salmon smolts from Chilko and Shuswap lakes, pooled over sample years 1950–1992, with species richness of other macroparasite communities from freshwater fish of the Fraser River drainage basin, bats, and birds while controlling for sampling effort (sample size). CH, Chilko Lake total parasite species richness; SH, Shuswap Lake total parasite species richness; SS1, SS2, SS3, SS4, parasite species richness of sockeye salmon smolts/presmolts from four different biogeoclimatic zones (Bailey and Margolis 1987); WH, parasite species richness of mountain whitefish from Tahtsa Lake, Fraser River drainage basin (Anonymous 1984); RT1, parasite species richness of rainbow trout from Tahtsa Lake, Fraser River drainage basin (Anonymous 1984); RT2, parasite species richness of rainbow trout from McGregor River, Fraser River drainage basin (Arai and Mudry 1983); KO1, parasite species richness of kokanee from Tahtsa Lake, Fraser River drainage basin (Anonymous 1984); KO2, parasite species richness of kokanee from Kenney Dam, Fraser River drainage basin (Anonymous 1984); KO3, parasite species richness of kokanee from McGregor River, Fraser River drainage basin (Arai and Mudry 1983); LS1, parasite species richness of longnose sucker from Kenney Dam, Fraser River drainage basin (Anonymous 1984); LS2, parasite species richness of longnose sucker from McGregor River, Fraser River drainage basin (Arai and Mudry 1983); CK, parasite species richness of chinook salmon juveniles from McGregor River, Fraser River drainage basin (Arai and Mudry 1983); DV, parasite species richness of Dolly Varden from McGregor River, Fraser River drainage basin (Arai and Mudry 1983); WS, parasite species richness of white sturgeon from Fraser River (Margolis and McDonald 1986); BT1, BT2, intestinal helminths of bats from two separate studies in the central United States (Lotz and Font 1985); DU, intestinal helminths of lesser scaup ducks (Bush and Holmes 1986); AV1, AV2, AV3, AV4, intestinal helminths of four different populations of American avocet (Edwards and Bush 1989).



population levels. The latter study most closely reflects the results of this study which found that the sockeye parasite community of Chilko Lake was significantly variable from year to year but exhibited no long-term trends. Although host densities other than that of sockeye were not available, comparison of parasite levels with sockeye densities, annual rainfall, and mean annual temperature did not indicate that these were systematically important to the parasite community.

Parasite relative abundances were consistent through time, even though absolute numbers were not. The most common, or core, parasites were tapeworms. Positive correlations between parasite species primarily involved tapeworms. This could be a result of similar life cycles and common hosts, or may merely arise from statistical associations, all being so common. *Proteocephalus* sp., *Eubothrium* sp., and *Diphyllobothrium ditremum* all use copepod intermediate hosts to transmit to sockeye smolts. Fluctuations in copepod population levels are liable to affect these species similarly. *Diphyllobothrium ditremum* and *Tetracotyle* sp. were also correlated in numbers. Both use smolts as second intermediate hosts and piscivorous birds as final hosts. Common final hosts include gulls (*Larus* spp.) and mergansers (*Mergus* spp.) (*Diphyllobothrium ditremum* host distribution: Becker and Brunson 1967; *Tetracotyle* sp. host distribution: Gibson 1996). Bailey and Margolis (1987) noted that Chilko Lake has Bonaparte's gulls (*Larus philadelphia*) and mergansers (*Mergus* spp.), which feed extensively on smolts gathered at the mouth of Chilko River in preparation for seaward migration, suggesting that these are likely final hosts for both parasites. Final host population fluctuations may similarly affect *Diphyllobothrium ditremum* and *Tetracotyle* sp., thus creating correlations between their prevalences and abundances. There is no evidence that parasite species are interfering with each other by competition, since all intercorrelations were positive.

The criteria for good biological tags are parasites that infect young salmon in the home system, differ in abundance among home systems, remain with the fish throughout time of study, are easily detectable, and do not cause large-scale selective mortality or change host behaviour (Bailey et al. 1989; Moser 1991). High mean prevalences of all three core species (>55%) make them easy to detect with a minimum sample size. Of all Chilko Lake annual samples, 76% were dominated by the three cestodes. These species were present in smolts every year and varied least in annual prevalence and mean intensity (<38 and <62% of the means, respectively). Low interannual variation is important because differences in prevalence and intensity alone are often used to discriminate between populations (Margolis 1965; Moser 1991). In addition to being easily detectable and consistently present, this study found that two of the tapeworms meet the second criterion for good biological tags: *Eubothrium* sp. and *Proteocephalus* sp. have significantly different levels of infection in Chilko Lake than in Shuswap Lake stocks. Unfortunately, those two core species that differed between the lakes (*Proteocephalus* sp. and *Eubothrium* sp.) do not remain with sockeye in the marine environment for more than a few months (Bailey et al. 1989), making their usefulness as tags short-lived. Although *Diphyllobothrium ditremum*, the other core species, remains with sockeye throughout its marine residence (Bailey et al. 1988), it does not differ in abundance between lakes. All three cestode species interfere with smolt migration through their effects on orientation, growth, or seawater acclimation (Becker and Brunson 1967; Boyce 1979; Boyce and Clarke 1983; Garnick and Margolis 1990). However, there is no evidence that these

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detrimental effects are not completely independent of home system (recall Moser's (1991) criteria of nonselective mortality). Other species that fulfilled the second criterion for biological tags, having different infection levels between lakes, were also relatively abundant as secondary species in the community: *Neoechinorhynchus* sp. and unidentified digenetic metacercariae (Table 2). However, the former makes a short-lived tag: *Neoechinorhynchus rutili* and *N. salmonis*' life cycles are both thought to be annual (Ching 1984; Bailey et al. 1989), persisting a minimum of 2–4 weeks in sockeye after entering seawater (Bailey et al. 1988). Unidentified digenetic metacercariae rely on sockeye as a second intermediate host. Since both identifiable digenetic metacercariae, *Tetracotyle* sp. and *Diplostomum* sp., survive sockeye marine residence (Bailey et al. 1988), it is presumed that unidentified digenetic metacercariae would similarly persist. Although the annual presence of both *Neoechinorhynchus* sp. and unidentified digenetic metacercariae is reliable (infecting smolts in 86 and 81% of years sampled, respectively), their mean annual prevalence is low (<16%) and their variation in prevalence high (>86%), making them harder to detect in small samples. There were no significant differences in prevalence or intensity between Chilko and Shuswap lakes for other parasite species present in both (Table 2). This includes parasites natural to sockeye that would have made more persistent tags.

The parasite fauna at Shuswap Lake is less rich (less diverse) than that at Chilko Lake. This may be because of the fewer number of years sampled at Shuswap Lake. Indeed, rare species at Chilko Lake, such as *Crepidostomum farionis* and the two copepod species, were not observed at Shuswap Lake. Such qualitative differences between Chilko and Shuswap lakes are unsuitable as tags because they occur so rarely at Chilko Lake and because they could represent a false difference based on insufficient number of years sampled.

The discriminant function for distinguishing between Chilko and Shuswap lakes based on the entire parasite community is not definitive, even though the lakes' mean parasite vectors were significantly different. Misclassification rates, although generally low, were quite high for Shuswap Lake fish alone. These misleading results are due to the violation of certain underlying assumptions in spite of data transformation. The assumption of equal variances between lakes was not met: this can lead to false rejection of the null hypothesis more often than the stated alpha level and is exacerbated by disproportionate sample sizes. The assumption of multivariate normality was also violated and is known to distort misclassification rates for individual groups. As Fig. 5 confirms, discriminant ability is limited because of low parasite loads and derives from quantitative, not qualitative, differences. Fish with high relative infections of certain species can be assigned with some confidence to one or the other lake: those infected with more than one nematode belong to Shuswap Lake; those highly infected with tapeworms originate from Chilko Lake.

Parasite species richness of Chilko Lake overall was 11, but averaged 8. Walther et al. (1995) argued that a major determinant of species richness estimates is sampling effort. Analysis of species accumulation curves suggests that our sample sizes were adequate to detect 95% of the parasite species present in the population sampled. Furthermore, sampling effort as indicated by sample size does not appear to be a consistent source of variation (Fig. 3). In spite of this unexplained variation in species richness, individuals remain distributed across species in a consistent manner: ranked species abundance plots, overall and for each year, best fit lognormal to log series species distribution models, models that represent communities of intermediate abundance (Magurran 1988).

In comparing Chilko Lake and Shuswap Lake parasite community data with other communities, we wanted to correct for sampling effort. The studies considered below were selected because they include cumulative parasite species counts and sample sizes for hosts. Fish species used in the following parasite species richness comparison were selected because they came from the Fraser River drainage basin, and in some cases were of a similar stage to sockeye of this study. Mammals and birds were also included for comparison, since it was felt that Kennedy et al.'s (1986) treatment of the subject is strengthened by inclusion of sampling effort. Fish parasite studies were selected from McDonald and Margolis' (1995) reference list.

The parasite community of sockeye smolts in both Chilko and Shuswap lakes is slightly lower in species richness than those of other sockeye smolt and presmolt populations of the Pacific Northwest (Bailey and Margolis 1987) when sampling effort is considered. The parasite species richness found in this study is also lower than that found in other juvenile and adult freshwater fish occurring in the Fraser River drainage basin, such as juvenile chinook salmon, adult kokanee, mountain whitefish, rainbow trout, Dolly Varden, longnose sucker (*Catostomus catostomus*), and white sturgeon (*Acipenser transmontanus*) (Fig. 6). Sockeye smolts in particular, and fish generally, are lower in parasite species richness than bats (*Eptesicus fuscus*) (Lotz and Font 1985), American avocets (*Recurvirostra americana*) (Edwards and Bush 1989), and lesser scaup ducks (*Aythya affinis*) (Bush and Holmes 1986) (latter studies were confined to intestinal helminths, underestimating overall parasite species richness), which Kennedy et. al (1986) attributed to differences in exposure, etc. Freshwater fish may host more parasite species than anadromous, juvenile sockeye studied herein because migratory fish are exposed to freshwater parasites for a shorter period of time than their lake-resident companions. Furthermore, because anadromous hosts tend to lose their freshwater parasites when they enter salt water, and in turn lose their marine parasites when they reenter freshwater, they may have lower numbers of parasite species adapted to exploit them.

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Fate of ^{60}Co and ^{134}Cs added to the hypolimnion of a Canadian Shield lake: accumulation in biota

G.A. Bird, W.J. Schwartz, and M. Motycka

Abstract: The addition of ^{60}Co and ^{134}Cs to the anaerobic hypolimnion of a thermally stratified Canadian Shield lake in summer initially isolated the exposure of biota to only zooplankton that undergo vertical migration into this region of the lake. Once the radionuclides became mixed throughout the water column with autumn turnover, other biota were exposed to the radionuclides. In general, ^{60}Co concentrations in biota were low because of the rapid loss to the sediments. Exceptions were for initial ^{60}Co concentrations in filter-feeders (cladocerans and clams) during autumn turnover. Concentrations of ^{134}Cs were higher in biota the following spring and summer, reflecting the tendency of ^{134}Cs to remain in the water column. Concentrations of ^{134}Cs still continued to increase in forage fish 1 year following the radionuclide addition. The addition of radionuclides to the hypolimnion resulted in higher concentrations in forage fish than when added to the epilimnion. Radionuclide concentrations in biota varied with taxon and were greatest in periphyton. This study demonstrates that radionuclides that may enter the bottom waters of a lake as a result of nuclear fuel waste disposal will be available to biota and result in a radiation dose to aquatic biota and to humans via the water–fish–human pathway.

Résumé : Dans une étude réalisée en été, nous avons ajouté du ^{60}Co et du ^{134}Cs à l'hypolimnion anaérobie d'un lac à stratification thermique du Bouclier canadien : seul le zooplancton migrant qui présente une migration verticale dans cette région du lac a d'abord été mis en évidence. Toutefois, après que les radionucléides se sont mélangés à toute la colonne d'eau, sous l'effet du renversement des eaux automnal, d'autres éléments du biote y ont été exposés. En général, les concentrations de ^{60}Co étaient faibles chez les organismes exposés, car ce radionucléide est passé rapidement dans les sédiments; il faut excepter ici les concentrations initiales de ^{60}Co mesurées chez les filtreurs (cladocères et bivalves) durant le renversement des eaux automnal. Quant aux concentrations de ^{134}Cs, elles étaient plus élevées le printemps et l'été suivants, ce qui concorde avec la tendance du ^{134}Cs à demeurer dans la colonne d'eau; les concentrations de ^{134}Cs étaient encore en hausse chez les poissons fourrage un an après l'ajout de ce radionucléide. Lorsque les radionucléides ont été ajoutés à l'hypolimnion, les concentrations mesurées chez les poissons fourrage ont été plus élevées que lorsque les marqueurs ont été introduits dans l'épilimnion. Les concentrations de radionucléides variaient selon le taxon; leur valeur était maximale dans le périphyton. Cette étude démontre que les radionucléides se retrouvant dans les eaux du fond d'un bassin lacustre par suite de l'élimination de déchets de combustible nucléaire peuvent être assimilés par les éléments du biote aquatique et déterminer la radioexposition de ceux-ci et des humains suivant la chaîne eau–poissons–humains.
[Traduit par la Rédaction]

Introduction

The operation of nuclear facilities such as generating stations, fuel reprocessing plants, and waste disposal operations has resulted in a continual release of radionuclides to the environment. Nuclear accidents can also result in radionuclide releases to the environment. For example, following the Chernobyl accident, ^{134}Cs and ^{137}Cs were the major radionuclides in atmospheric fallout in Europe (Santschi et al. 1990). ^{135}Cs is also a concern to nuclear fuel waste disposal because of its long half-life, mobility in the environment, and its tendency for uptake by biota (Elderfield and Truesdale 1980). Other relatively short-lived radionuclides such as ^{60}Co ($t_{1/2}$ = 5.3 years), ^{134}Cs ($t_{1/2}$ = 2.1 years), and ^{137}Cs ($t_{1/2}$ = 30.2 years) are abundant fission products that are present in low-level aqueous waste discharges from nuclear facilities and may be taken up by biota (Ophel and Fraser 1971; Kolehnainen 1972). In the Canadian Nuclear Fuel Waste Management Program (NFWMP), ^{90}Sr, ^{137}Cs, ^{134}Cs, and ^{60}Co are the most important radionuclides in terms of the potential dose to humans during the operation of a disposal facility (Russell 1993).

The Canadian Nuclear Fuel Waste Management (NFWMP) is proposing the disposal of nuclear fuel waste deep underground in the crystalline rock of the Canadian Shield using natural and engineered barriers to isolate the waste from the biosphere (AECL 1994). A possible scenario would be for such radionuclides to be released from an underground vault and to be transported to the surface with deep groundwater flow along a fracture in the geosphere to a lake or wetland in the future. A quantitative assessment of the plausible fate of radionuclides discharged to surface waters with groundwater flow has been undertaken by the NFWMP (Bird et al. 1992, 1993*a*, 1995; AECL 1994). In a previous study, we (Bird et al. 1995) reported on the fate of ^{60}Co and ^{134}Cs added to the anaerobic hypolimnion of a shield lake to simulate the NFWMP scenario where the radionuclides enter the bottom of a lake with deep groundwater discharge and compared their behaviour with that when the radionuclides were added to the

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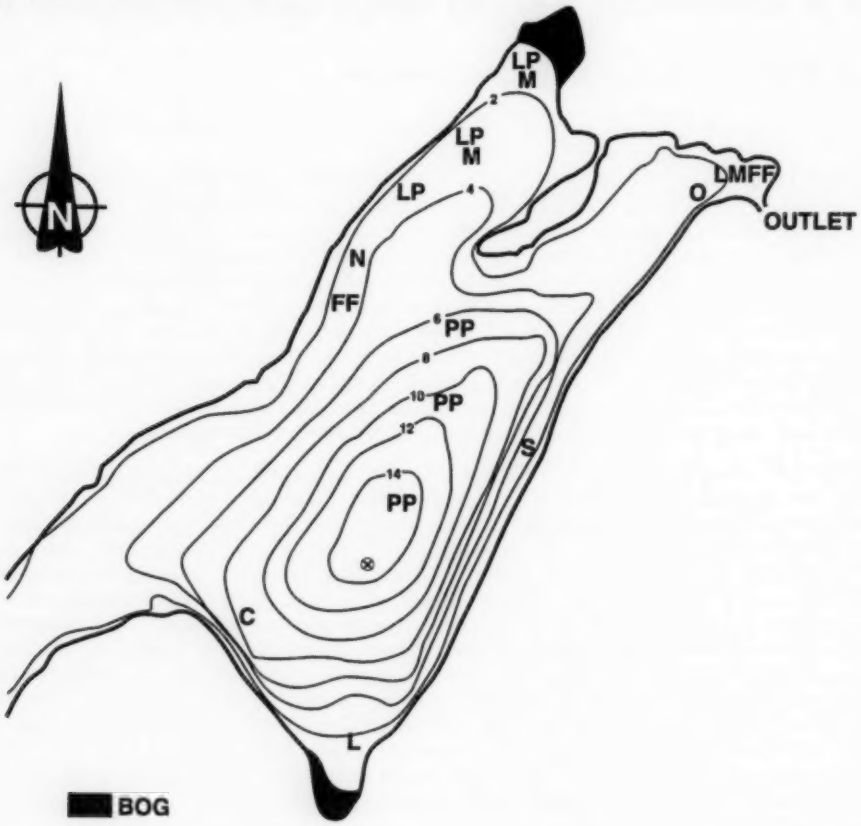
Fig. 1. Bathymetric map of L226N illustrating the location of the radionuclide addition and sampling locations. ⊗, radionuclide addition; N, S, and O, clam cages, north, south, and outlet bays, respectively; PP, pelagic periphyton; LP, littoral periphyton; M, macrophytes; C, chironomid larvae; L, leeches; FF, forage fish.



epilimnion of the same lake (Hesslein et al. 1980; Hesslein 1987).

The radionuclides ^{60}Co and ^{134}Cs were added to the lake because they behave quite differently in the way in which they are lost from the water and bound to sediment. Co is a divalent cation that readily binds to suspended organic particles, which are subject to sedimentation (Parker and Hasler 1969; Hesslein et al. 1980; Santschi et al. 1986; Hesslein 1987; Bird et al. 1995). The behaviour of Co is particularly sensitive to changes in redox potential. Under moderately reducing conditions, Co is released from sediment as Co^{2+} and is precipitated as CoS in the presence of sulfide (Balistrieri et al. 1992; Wallman 1992). Cs is a monovalent cation that does not readily bind to suspended organic particles but is lost from the water column by direct sorption to bottom sediments (Hesslein et al. 1980; Santschi et al. 1986; Hesslein 1987). Presumably, binding is to clay particles in organic-bottom sediments because Cs readily binds to clay particles (Alberts et al. 1989; Bird and Evenden 1994). Both these radionuclides are expected to be taken up by biota.

The present study tested the hypothesis that ^{60}Co and ^{134}Cs added to the anaerobic hypolimnion of a shield lake will be available for uptake by biota and that uptake will be similiar to that following epilimnetic addition.

Materials and methods

Study site

The Experimental Lakes Area (ELA) is located in northwestern Ontario on the Canadian Shield and is described in Brunskill and Schindler (1971) and Cleugh and Hauser (1971). Lake 226 (L226) is a small (16.1 ha) dimictic, double-basin, headwater lake naturally divided into two basins by a shallow channel (Findlay and Kasian 1987). The northeast basin (L226N) has an area of 8.3 ha with a maximum depth of 14.7 m (Fig. 1). No permanent stream flows into L226; it is fed by direct precipitation and runoff. The outflow is located at the north end of the northeast basin. L226 was the site of a fertilization study from 1973 to 1980, L226N receiving annual inputs of carbon (3.67 $g \cdot m^{-2}$), nitrogen (1.92 $g \cdot m^{-2}$), and phosphorus (0.34 $g \cdot m^{-2}$) (Findlay and Kasian 1987). After nutrient inputs stopped, the primary production of L226N quickly returned to prefertilization levels (Shearer et al. 1987). On July 25, 1977, 4.5 GBq each of ^{60}Co and ^{134}Cs and between 4.5 and 13 GBq of ^{75}Se, ^{203}Hg, ^{85}Sr, ^{59}Fe, and ^{65}Zn were added to the epilimnion of both basins (Hesslein et al. 1980; Hesslein 1987). A becquerel (Bq) is a disintergration per second and is equivalent to 27 pCi.

For our experiment, 4.6 GBq of ^{60}Co (as $CoCl_2$), 4.6 GBq of ^{134}Cs (as CsCl), and 370 GBq of ^{3}H (as ^{3}HHO) were added as an acidified solution (4 mL of 6 N HNO_3 in 246 mL of distilled water) to the water column at 12 m depth of the anaerobic hypolimnion of L226N (Fig. 1) on August 2, 1989. The injection system and the radionuclide addition are described in Bird et al. (1993*b*, 1995).

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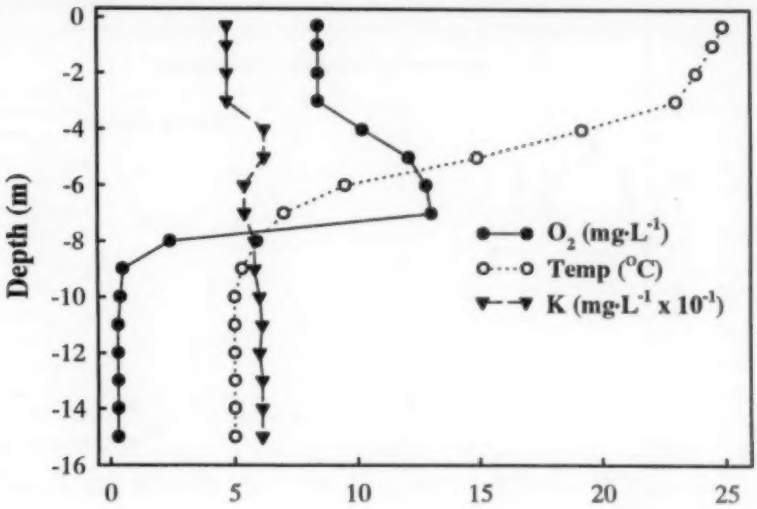
Fig. 2. Depth profiles for oxygen and K concentrations and water temperature in L226N during the radionuclide addition on August 2, 1989.



Biota

Sampling of biota coincided with sampling of the water, sediments, and other parameters reported by Bird et al. (1995). Biota sampled on a routine basis included clams (*Anodonta grandis grandis*), periphyton, macrophytes, zooplankton (primarily *Holopedium gibberum*, *Chaoborus flavicans*, and *Chaoborus punctipennis*), leeches, minnows (pearl dace (*Margariscus margarita*), northern redbelly dace (*Phoxinus eos*), and fathead minnow (*Pimephales promelas*)), and slimy sculpin (*Cottus cognatus*). Other taxa were collected opportunistically. All biota were stored on dry-ice, except chironomid larvae which were stored in 70% ethanol, until being returned to the laboratory where they were stored in a freezer until processed.

Clams were collected from ELA Lake 938 on June 28, 1989, and placed into cages (1.2 m long × 0.6 m wide × 0.2 m high) submerged into L226N at 2 m depth at the three locations illustrated in Fig. 1. Before being placed into the cages, their wet weights and maximum lengths were recorded and each specimen was identified with a number painted on the shell with nail polish. To determine initial radionuclide concentrations, five specimens were sacrificed prior to being placed into L226N. On each sampling occasion, five specimens were collected from each cage. To process the clams for radionuclide determination in the laboratory, specimen wet weights and lengths were determined and the bodies were removed from their shells, dried at 60°C (shell and body tissues), and reweighed. Body tissues were then ground in a clean air hood and placed into a 7-mL vial to a standard geometry for gamma counting.

Zooplankton were collected by taking vertical net hauls from a raft located above the point of radionuclide injection. Samples were collected at dusk from a depth of 12 m (2 m above the bottom) to avoid contaminating the samples with bottom sediments. A 1-m-diameter net of 250-µm mesh was used to maximize collection of *Chaoborus* and *H. gibberum* and to minimize the collection of copepods. The contents of each haul were strained through a 250-µm mesh and placed into a 250-mL wide-mouth sample bottle. Zooplankton were collected until six sample bottles were filled on each sampling occasion. In the laboratory, samples were thawed and *Chaoborus* were manually separated from the remaining zooplankton. The remainder of the sample, which was primarily *H. gibberum*, was filtered through a 1-µm mesh to remove the fluids. The fluids were processed as described for water samples by Bird et al. (1995). Both the *Chaoborus* and *H. gibberum* were dried at 60°C, placed in preweighed 7-mL vials (to a standard geometry), reweighed, and counted for gamma activity.

Periphyton, mostly algae, were collected by scraping rocks and submerged logs found along the shoreline (<0.5 m depth) into scintillation vials. Samples were also occasionally collected from the pelagic zone by scraping periphyton from boards (floats) and ropes attached to our sediment traps. Ideally, five scintillation vials of algae were collected on each sampling occasion, but this was not always possible. Periphyton were counted in the scintillation vials, after which the samples were dried (60°C) and weighed.

Macrophytes (*Dulichium arundinaceum*, *Carex lanuginosa*, *Eriocaulon septangulare* – *Lobelia dortmanna*, and *Nuphar variegatum*) were collected along the shoreline (<1 m depth) by hand, except for *Potamogeton amplifolius* which was collected with the use of a garden rake from deeper water, and each species was placed in appropriately labelled plastic bags. Because of their similarity in appearance, *E. septangulare* and *L. dortmanna* were pooled. Processing macrophytes involved taking a composite subsample of plant material representing the nonroot portion of the plant. The composite was gently washed with tap water to remove sediment particles, dried at 60°C, ground in a Wiley mill, and a weighed amount analyzed for gamma activity. Where samples allowed, roots, stems, leaves, and flowers were analyzed separately.

Leeches were collected from pieces of beef liver anchored under a rock in the shallows. Most of the leeches were collected near the lake outlet, but specimens were also collected from baits at other sites (Fig. 1) or opportunistically while collecting other samples.

Aquatic insects, mainly Odonata larvae, and amphipods were collected by passing a net bag (~800-µm mesh) over the surface sediments and sorting through the detritus in the field. Chironomid larvae were collected from a depth of about 7 m using a stainless steel Ekman grab (15.2 cm long × 15.2 cm wide × 22.9 cm high). This involved taking about 25 Ekman samples, sieving (800-µm sieve) them, and then collecting the larvae. These larvae were pooled into a single sample and preserved in 70% ethanol.

Forage fish (minnows and slimy sculpin) were collected from the shallows (~1 m depth) and from deeper waters (~4–6 m depth) using standard minnow traps baited with liver. For reference, minnows were also collected from nearby Lake 114. In the laboratory, the minnows were sorted to species, and each specimen was measured for total length. A pooled sample for each species was dried, weighed, ground, and analyzed for gamma activity.

Bioaccumulation factors and radiological and statistical analyses

Bioaccumulation factors (BAFs, litres per kilogram dry weight (dw))

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Fig. 3. Mean (±SD) percent of the total ^{60}Co and ^{134}Cs added to L226N remaining in the water column over the 358-day study period at the station where the radionuclides were added to the anaerobic hypolimnion. The solid bar indicates ice cover.

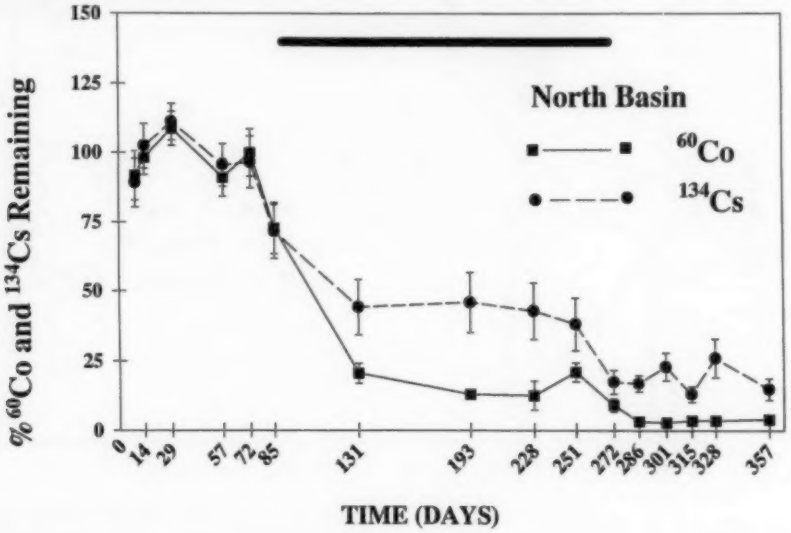
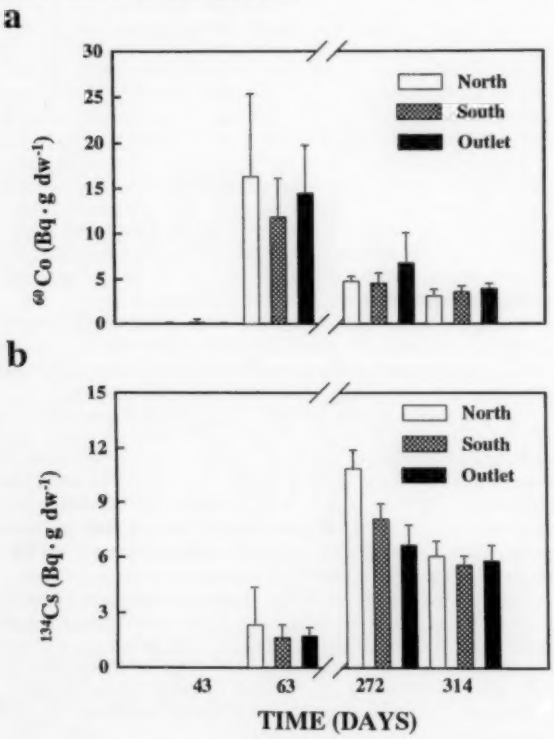


Fig. 4. Mean (±SD) concentrations of (*a*) ^{60}Co and (*b*) ^{134}Cs in clams (*A. grandis grandis*) in cages in the north, south, and outlet bays of L226N over the study period.



were calculated as the ratio of radioactivity in the organism (becquerels per kilogram dry weight) to the radioactivity in unfiltered lake water (becquerels per litre).

All samples were analyzed using a high-purity germanium detector in conjunction with a Nuclear Data 6700 computer/multichannel analyzer to detect gamma emissions for ^{134}Cs at 796-keV and ^{60}Co at 1332-keV energies. Counts were for samples corresponding to standard geometries, assuming uniform distribution of the radioactivity throughout the sample.

Statistical analysis of data was by two-way ANOVA using the SAS GLM program (SAS Institute Inc. 1985). Means are quoted ±1 SEM unless specified otherwise. All concentrations are arithmetic means and all BAFs are geometric means. Uptake rates and loss rates for ^{60}Co and ^{134}Cs in biota were determined by regression of the ln-transformed data. Probabilities at or below $P = 0.05$ are deemed significant. All radionuclide data were back-dated to August 2, 1989, to account for radioactive decay.

Results

Water

The K concentration in the lake remained about 0.57 mg·L^{-1} throughout the study period (Fig. 2). Detailed depth–time profiles for the radionuclides and oxygen concentrations, water temperature, and sedimentation over the study period are presented in figs. 3 and 7 of Bird et al. (1995). When the radionuclides were added to the lake, the hypolimnion was anaerobic below 9 m depth, the anaerobic water expanding upward to 8 m depth as summer progressed.

Little or no loss of ^{60}Co and ^{134}Cs occurred from the water over the first 72 days of the study (Fig. 3) while the radionuclides were confined to the anaerobic hypolimnion (figs. 3*a* and 3*b* of Bird et al. 1995). Autumn turnover resulted in the mixing of the radionuclides throughout the water column. This resulted in ^{60}Co being rapidly lost from the water whereas ^{134}Cs was lost at a slower rate (Fig. 3). One year (day 357) following the radionuclide addition, only about 4% of the initial ^{60}Co inventory and 15% of the ^{134}Cs inventory remained in the water column of L226N.

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Table 1. Geometric mean BAFs (L·kg dw^{-1}) calculated for ^{60}Co and ^{134}Cs in biota over the 1-year period following the radionuclide addition to L226N.

	^{60}Co			^{134}Cs		
Biota	GM	GSD	n	GM	GSD	*n*
Anodonta grandis grandis	3 600	1.7	57	1 500	3.155	55
Zooplankton (pooled)	100	4.1	15	140	5.6	15
Holopedium gibberum	190	3.0	8	260	3.2	8
Chaoborus	68	7.3	7	46	4.0	7
Periphyton littoral	24 000	4.2	10	10 100	1.8	10
Periphyton pelagic	64 000	3.5	15	20 000	2.5	18
Macrophytes (pooled)	2 000	6.5	24	1 400	5.5	25
Nuphar variegatum	1 900	2.8	5	4 400	4.5	5
Carex lanuginosa stem	460	3.7	7	370	2.7	7
C. lanuginosa root	1 500	5.1	5	690	2.9	5
Dulichium arundinaceum	870	2.2	6	460	3.1	6
D. arundinaceum root	1 900	5.1	4	1 900	5.0	4
Eriocaulon septangulare – Lobelia dortmanna	1 200	8.7	6	1 400	3.1	4
Potamogeton amplifolium	37 000	1.8	2	7 700	1.7	2
Forage fish	530	2.4	41	140	4.0	82
Cottus cognatus	440	2.8	3	1 600	1.9	9
Margariscus margarita	260	1.9	13	480	2.5	33
Phoxinus eos	870	1.7	15	4 000	2.3	25
Pimephales promelas	990	1.5	8	4 400	2.3	13
Hyalella azteca	6 600		1			
Odonata nymphs	20 000		1	1 600		1
Leeches	1 900	1.6	3	340	1.3	3
Tadpoles	20 000	1.2	2	15 000	1.0	2
Leptophebia nymphs	2 400		1	8 300		1
Chironomid larvae	5 900		1	1 100		1

Note: *n* is the number of replicates. Samples with nondetectable radionuclide levels were excluded from the distributions.

Biota

Prior to the radionuclide addition, ^{60}Co and ^{134}Cs were not detectable in biota from L226N with the exception of low levels found in pearl dace and the roots of *E. septangulare* / *L. dortmanna* (see below). For biota, other than zooplankton, no uptake of the radionuclides occurred until autumn turnover because the radionuclides were confined to the hypolimnion. Dry weight was 9% of wet weight (ww) for soft tissues of clams, 6% for periphyton, 5% for *Chaoborus*, 12% for leeches, 7% for tadpoles, 4–30% for macrophytes, and 25% for forage fish. These percentages can be used to convert radionuclide concentrations and BAFs to wet weight values.

Clams

Maximum ^{60}Co concentrations in clams of 14.2 ± 7.0 Bq·g dw^{-1} were found in November (day 90) when the lake was mixing (Fig. 4*a*). There was no difference in the ^{60}Co concentrations in clams with location in the lake. Concentrations decreased significantly ($P < 0.05$) over the winter (day 272) and the following summer (day 314). In comparison with ^{60}Co, ^{134}Cs concentrations in clams in November were low (1.9 ± 1.4 Bq·g dw^{-1}), with maximum concentrations of 8.5 ± 2.1 Bq·g dw^{-1} being reached the following spring (day 272, Fig. 4*b*). These concentrations were similar to those observed for ^{60}Co in spring and were elevated in specimens ($P < 0.05$) collected from the north bay (Fig. 4*b*). Concentrations of ^{134}Cs declined slightly as summer progressed (day 314) to levels similar to ^{60}Co. BAFs between water and soft tissues for the period following autumn mixing of the radionuclides in the water column were 3600 $\times/\div$ 1.7 (geometric mean (GM) $\times/\div$ geometric standard deviation (GSD)) for ^{60}Co and 1500 $\times/\div$ 3.1 for ^{134}Cs (Table 1).

Zooplankton

Uptake of the radionuclides by *Chaoborus* and *H. gibberum* occurred immediately (Fig. 5). In *Chaoborus*, ^{60}Co concentrations peaked at about 6 Bq·g dw^{-1} on day 14, with levels tending to decrease thereafter (Fig. 5*a*). Much lower ^{60}Co concentrations were found in *Chaoborus* in spring (day 272) than during the previous autumn. Levels of ^{60}Co in *Chaoborus* fluctuated as summer progressed, with nondectable levels being recorded on day 358. In the case of ^{134}Cs, a maximum concentration of 7.5 Bq·g dw^{-1} in *Chaoborus* was observed on day 56. Similar ^{134}Cs concentrations were observed in autumn (day 84) and the following spring, after which they decreased. Over the study, BAFs of 68 $\times/\div$ 7.3 for ^{60}Co and 46 $\times/\div$ 4.0 for ^{134}Cs in *Chaoborus* were recorded (Table 1).

Concentrations of radionuclides were considerably higher in *H. gibberum* than in *Chaoborus* (Fig. 5), with maximum ^{60}Co (30 Bq·g dw^{-1}) and ^{134}Cs (32 Bq·g dw^{-1}) concentrations occurring during autumn turnover (day 84). Radionuclide concentrations were much lower in *H. gibberum* the following spring and were similar to those in *Chaoborus*. Low levels of radioactivity were measured in *H. gibberum* over the summer. Over the study, BAFs of 190 $\times/\div$ 3.0 for ^{60}Co and 260 $\times/\div$ 3.2 for ^{134}Cs were recorded in *H. gibberum* (Table 1).

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Fig. 5. Mean (±SD) concentrations of ^{60}Co and ^{134}Cs in (*a*) *Chaoborus* and (*b*) *H. gibberum* in L226N over the study period.

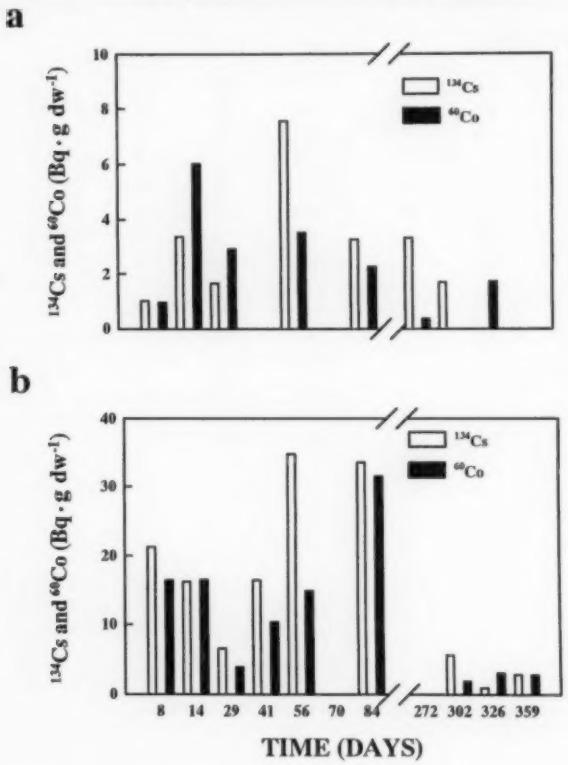
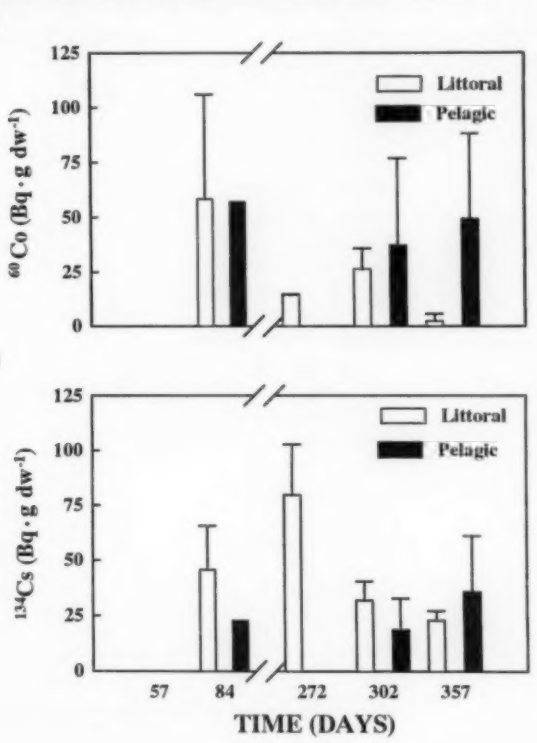


Fig. 6. Mean (±SD) concentrations of (*a*) ^{60}Co and (*b*) ^{134}Cs in littoral and pelagic periphyton in L226N over the study period.



Periphyton

Radionuclides were not observed in periphyton until autumn turnover (day 85) when about 58 ± 18 Bq $^{60}Co \cdot g\ dw^{-1}$ and 42 ± 8 Bq $^{134}Cs \cdot g\ dw^{-1}$ were observed in the samples (Fig. 6*a*). There was no difference in the ^{60}Co concentration in pelagic samples and littoral samples in the autumn (day 85), but the ^{134}Cs concentrations in pelagic samples were only about half ($P > 0.05$) those in littoral samples (Fig. 6*b*). The ^{60}Co concentration in littoral periphyton decreased fivefold over the winter (day 272) and then increased slightly following spring turnover. Low levels of ^{60}Co were found in littoral periphyton on day 357. In contrast, levels of ^{60}Co in pelagic samples tended to increase over the summer and were higher than levels in littoral samples. In comparison with ^{60}Co, ^{134}Cs levels in littoral periphyton were higher in spring (day 272) than in autumn (day 85), but decreased over the summer. As for ^{60}Co, ^{134}Cs levels in pelagic periphyton increased over the summer. Over the study, BAFs for pelagic periphyton were 64 000 $\times/\div$ 3.5 for ^{60}Co and 20 000 $\times/\div$ 2.5 for ^{134}Cs whereas BAFs for littoral periphyton were 7700 $\times/\div$ 26.6 for ^{60}Co and 10 100 $\times/\div$ 1.8 for ^{134}Cs.

Macrophytes

Unfortunately, not all macrophyte species were available on each sampling occasion, so complete temporal trends for radionuclides in each species are not available. Radionuclides were not detected in the macrophytes prior to the radionuclide addition, with the exception of *E. septangulare – L. dortmanna* roots, which had ^{60}Co levels of about 0.7 Bq·g dw^{-1} (Table 2).

Yellow water lily (*N. variegatum*) was collected only on days 85 and 358. Concentrations of ^{60}Co showed a marked decrease by day 358 whereas ^{134}Cs concentrations increased threefold between days 85 and 358 (Table 2). Concentrations of ^{60}Co and ^{134}Cs in both nonroot tissues and roots of *C. lanuginosa* increased from autumn to spring and then decreased markedly for ^{60}Co and more gradually for ^{134}Cs. In *D. arundinaceum*, concentrations of about 3 Bq·g dw^{-1} for ^{60}Co and about 1 Bq·g dw^{-1} for ^{134}Cs were observed in autumn. ^{60}Co concentrations decreased by a factor of 4 by day 358 whereas ^{134}Cs concentrations increased slightly. For *E. septangulare / L. dortmanna*, radionuclide concentrations in nonroot tissues increased about fivefold between autumn and day 358 and by a factor of 2 in their roots. In the case of *P. amplifolius*, concentrations of 26.7 ± 1.2 Bq·g dw^{-1} for ^{60}Co and 13.0 ± 1.3 Bq·g dw^{-1} for ^{134}Cs were observed late in the study (days 315 and 358). When all samples for each taxon collected between days 85 and 358 were pooled, ^{60}Co concentrations in nonroot tissues were in the order *P. amplifolius* > *E. septangulare / L. dortmanna* > *N. variegatum* > *D. arundinaceum* > *C. lanuginosa* whereas ^{134}Cs concentrations were in the order *P. amplifolius* = *N. variegatum* > *E. septangulare – L. dortmanna* > *D. arundinaceum* > *C. lanuginosa*. Overall, BAFs of 2000 $\times/\div$ 6.5 for ^{60}Co and 13 400 $\times/\div$ 5.5 for ^{134}Cs were recorded in the nonroot portion of all the macrophyte

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species pooled. Bioaccumulation factors for individual species are presented in Table 2.

Fish

Radionuclides were not detected in forage fish (minnows and slimy sculpin) until after autumn turnover (Fig. 7). An exception is ^{134}Cs found in pearl dace collected prior to the radionuclide addition (Fig. 7). In general, levels of ^{60}Co in minnows were low (<0.3 Bq·g dw^{-1}). In comparison, ^{134}Cs levels increased from 1.2 ± 1.2 Bq·g dw^{-1} on day 85 to about 9.3 ± 1.1 Bq·g dw^{-1} on day 358. Thus, almost all the radioactivity in the fish was attributable to ^{134}Cs, the ^{60}Co concentrations being low or nondetectable. Trends in radionuclide concentrations in pearl dace, redbelly dace, fathead minnow, and slimy sculpin were similar, with levels of ^{134}Cs tending to increase markedly the following summer. The BAFs for all the forage fish pooled together were 530 $\times/+$ 2.4 for ^{60}Co and 1400 $\times/+$ 4.0 for ^{134}Cs over the study period; values for individual species are presented in Table 1.

Other biota

We were unsuccessful in collecting leeches in the autumn of 1989, but specimens collected on days 284 and 301 had <1 Bq·g dw^{-1} for both ^{60}Co and ^{134}Cs whereas nondetectable levels were recorded on day 358. Bioaccumulation factors in leeches were 1900 $\times/+$ 1.6 for ^{60}Co and 360 $\times/+$ 1.3 for ^{134}Cs. Tadpoles collected on days 301 (May 31) and 328 (June 27) had mean concentrations of 5.9 Bq·g dw^{-1} for ^{60}Co and 3.5 Bq·g dw^{-1} for ^{134}Cs. Bioaccumulation factors in tadpoles were 20 000 $\times/+$ 1.2 for ^{60}Co and 15 000 $\times/+$ 1.0 for ^{134}Cs. Chironomid larvae collected on day 327 had ^{60}Co and ^{134}Cs concentrations of 2.0 and 1.4 Bq·g dw^{-1}, respectively, and BAFs of 5900 for ^{60}Co and 1100 for ^{134}Cs. On day 272, *Hyallela azteca*, Odonata larvae, and *Leptophlebia* nymphs had ^{60}Co concentrations of <0.5, 5.1, and 1.7 Bq·g dw^{-1}, respectively, and ^{134}Cs concentrations of 15.6, 11.3, and 11.3 Bq·g dw^{-1}, respectively; the corresponding BAFs are presented in Table 1.

Table 2. Concentration (mean ± SD) of ^{60}Co and ^{134}Cs (Bq·g dw^{-1}) in macrophytes following their addition to the hypolimnion of L226N.

	Days postaddition				
Plant	57	85	302	315	357
Nuphar variegatum					
Nonroot		(2)			(3)
^{60}Co	0	7.2±8.3			18.8±14.9
^{134}Cs	0	6.5±0.01			0.9±0.9
Carex lanuginosa					
Nonroot		(4)	(1)	(1)	(1)
^{60}Co	0	1.0±0.6	1.7	0.7	0
^{134}Cs	0	0.8±0.9	1.8	1.6	1.3
Root		(2)	(1)	(1)	(1)
^{60}Co	0	1.7±0.2	3.1	0.4	3.5
^{134}Cs	0	1.3±0.2	2.6	2.3	3.4
Dulichium arundinaceum					
Nonroot		(4)			(2)
^{60}Co	0	2.9±1.9			0.7±0.3
^{134}Cs	0	1.4±0.6			2.1±0.3
Root		(2)			(2)
^{60}Co	0	1.4±0.1			3.6±1.0
^{134}Cs	0	2.2±0.2			9.3±2.2
Eriocaulon septangulare – Lobelia dortmanna					
Nonroot		(2)		(1)	(3)
^{60}Co	0	2.3±0.2		15.6	13.9±5.2
^{134}Cs	0	3.2±0.1		17.2	13.1±10.1
Root		(2)			(2)
^{60}Co	0	3.4±0.7			7.9±0.3
^{134}Cs	0	3.5±0.4			5.2±5.6
Potamogeton amplifolius					
Nonroot				(1)	(1)
^{60}Co	0			27.6	25.9
^{134}Cs	0			14.8	13.0

Note: Number of samples counted given in parentheses.

Discussion

The radionuclides ^{60}Co and ^{134}Cs were added to the anaerobic hypolimnetic waters of L226N to determine whether their ultimate fate is the same as when added to the epilimnion of the same lake. Addition was to an anaerobic hypolimnion because lakes with an anaerobic hypolimnion are common on the Canadian Shield and because we know how these radionuclides would behave in an aerobic hypolimnion, i.e., they would be lost to bottom sediments and hence would not be as available for uptake by biota as when added to the epilimnion. The accumulation of radionuclides in biota is a function of uptake, retention, and elimination from the organism. Each trophic level is a source of contaminants when they are passed on to the next trophic level. A major fraction of contaminants in the biotic community may be associated with plankton and benthic algae. This appears to be the case in L226N because accumulation is greater in algae per unit weight than in other biota and because algae may form a large fraction of the total biomass in L226N, which has a poorly developed littoral macrophyte fauna (M. Turner, Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Man., personal communication). However, retention tends to be greater in larger organisms than in smaller organisms (Rowan and Rasmussen 1994; Ugedal et al. 1995). Adsorption and absorption are the important mechanisms for radionuclide accumulation by algae and macrophytes, but the food chain is more important for uptake by fish (Thomann 1981; Harrison et al. 1990).

Only the zooplankton *H. gibberum* and *Chaoborus* accumulated radioactivity during the summer of addition when the radionuclides were confined to the anaerobic hypolimnion. These two species undergo diurnal vertical migration from the sediments into the water column at dusk, returning to the sediments at dawn. Thus, they were exposed to radionuclides in the hypolimnion immediately whereas exposure of other biota was delayed until the radionuclides became distributed throughout the water column with autumn turnover. Much higher concentrations were found in *H. gibberum* than in *Chaoborus*, possibly because of differences in feeding behaviour. *Holopedium gibberum* is a filter-feeder and is reported to feed on bacteria on iron particles at the oxic–anoxic water interface (Yan and Mackie 1990). High levels of radioactivity in *H. gibberum* may reflect its feeding on bacteria and other

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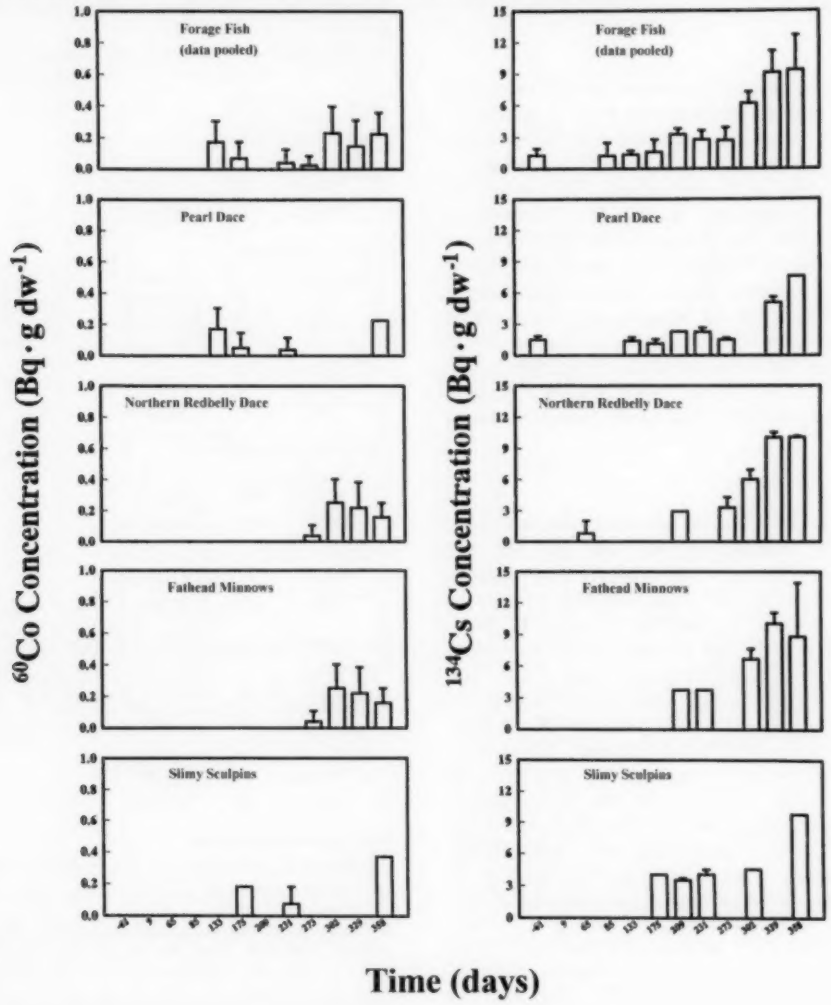
Fig. 7. Mean (±SD) concentrations of ^{60}Co and ^{134}Cs in forage fish in L226N over the study period.



small particles in the hypolimnion, although this may also be due to adsorption to its gelatinous case. In comparison, *Chaoborus* is a predator that feeds on other smaller zooplankton. Initial low levels of radioactivity in *Chaoborus* may be the result of feeding on uncontaminated prey outside the anaerobic hypolimnion.

Once the lake had mixed, the highest levels of radioactivity were found in the periphyton. Rapid accumulation of radioactivity by algae has been reported previously (Nucho and Baudin 1986; Lindner et al. 1989). In this respect, peak concentrations of ^{60}Co in periphyton occurred in autumn and decreased over the winter, similar to the loss of ^{60}Co from water. However, over the summer, periphyton ^{60}Co concentrations increased despite low concentrations in water whereas ^{134}Cs concentrations decreased from the peak values observed in spring.

Clams are used to monitor contaminant levels in aquatic ecosystems (Heit et al. 1980) because they sample large volumes of water and accumulate contaminants. Consistent with this, clams accumulated relatively high concentrations of the radionuclides (Fig. 4) compared with the other biota. Initially, more ^{60}Co than ^{134}Cs was accumulated by clams, which may reflect the fact that ^{60}Co is particle bound whereas ^{134}Cs tends to remain in solution (Hesslein et al. 1980; Hesslein 1987; Bird et al. 1995). Over the winter, ^{60}Co concentrations in clams decreased, and by spring, ^{60}Co and ^{134}Cs concentrations were nearly equal. Higher ^{134}Cs concentrations in spring probably reflect the higher ^{134}Cs concentrations remaining in the water. Ophel and Fraser (1971) also reported high concentrations of ^{60}Co in clams (*Elliptio* sp.).

Only trace amounts of ^{60}Co and ^{134}Cs were found in leeches. High concentrations were found in tadpoles, which probably reflect their feeding on detritus. Ophel and Fraser (1971) also found high ^{60}Co concentrations in tadpoles.

Chironomids are ubiquitous in aquatic systems and form a major component of the diet of many fish species, although this was not investigated in L226N. In our study, ^{60}Co (2.2 Bq·g dw^{-1}) and ^{134}Cs (3.1 Bq·g dw^{-1}) concentrations in

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Table 3. Uptake rates, k (day^{-1}), for ^{134}Cs in lakes at the ELA, northwestern Ontario, and for ^{137}Cs by freshwater fish as a result of the Chernobyl accident.

Fish	Lake	Nuclide	k	SE	R^2	Reference
All minnows	L226N, hypolimnion	^{134}Cs	0.009	0.0005	0.89	Present study
Fathead minnow	L226N, hypolimnion	^{134}Cs	0.009	0.013	0.89	Present study
Redbelly dace	L226N, hypolimnion	^{134}Cs	0.016	0.003	0.69	Present study
Pearl dace	L226N, hypolimnion	^{134}Cs	0.008	0.0009	0.86	Present study
Slimy sculpin	L226N, hypolimnion	^{134}Cs	0.005	0.003	0.81	Present study
Fathead minnow	L226N, epilimnion	^{134}Cs	0.011	0.0017	0.57	R. Hesslein, personal communication
Pearl dace	L226N, epilimnion	^{134}Cs	0.003	0.0006	0.51	R. Hesslein, personal communication
Slimy sculpin	L226N, epilimnion	^{134}Cs	0.005	0.0018	0.46	R. Hesslein, personal communication
Fathead minnow	L224, epilimnion	^{134}Cs	0.017	0.006	0.45	Harrison et al. 1990
Lake trout	L224, epilimnion	^{134}Cs	0.0002	0.000	0.79	Harrison et al. 1990
Brown trout	Norway	^{137}Cs	0.041	0.004	0.98	Brittain et al. 1991
Arctic char	Hoysjoen, Norway	^{137}Cs	0.006	0.005	0.26	Ugedal et al. 1995
Brown trout	Hoysjoen, Norway	^{137}Cs	0.093	0.019	1.0	Ugedal et al. 1995
Trout and char	Lake Vekteren, Norway	^{137}Cs	0.009	0.002	0.98	Lonvik and Koksvik 1990
Artic char	Swedish lakes	^{137}Cs	0.014	0.007	0.59	Hammar et al. 1991
Trout	Swedish lakes	^{137}Cs	0.035	0.016	0.82	Hammar et al. 1991
Perch	Swedish lakes	^{137}Cs	0.103	0.041	0.86	Håkanson et al. 1992
Pike	Swedish lakes	^{137}Cs	0.010	<0.002	0.86	Håkanson et al. 1992
Pike	NPP Cooling Pond		0.005			Kryshev 1995

Note: Hypolimnion, hypolimnion addition; epilimnion, epilimnion addition.

chironomid larvae were about an order of magnitude lower than those of the sediment (27 Bq·g dw^{-1} for ^{60}Co and 24 Bq·g dw^{-1} for ^{134}Cs) from which they were collected. This is in contrast with the findings of Gerking et al. (1976), who reported similar concentrations in larvae and sediment. Also, some chironomid larvae have been reported to feed selectively on algae in sediments (Kajak and Warda 1968) and therefore should have contaminant concentrations greater than those in the surrounding sediment. Co tends to be slightly higher in invertebrates than in water, but much less than in algae and macrophytes (Reichle et al. 1970).

Levels of radionuclides were higher in submerged macrophytes than in emergents and increased with time, a trend also observed by others (Ophel and Fraser 1971). Ophel and Fraser (1971) found that ^{60}Co concentrations in macrophytes were in the order submerged forms with finely divided structure > other submergents > floating leaf species (e.g., *Nuphar*) > emergents. The highest concentrations in our study were found in *P. amplifolius*, possibly because of its large surface area and because of radionuclide uptake by the epiphytic community growing on its leaves. Concentrations in macrophytes were generally higher 1 year after the addition (day 358) than during autumn turnover (day 85) when the radionuclides first became available to the macrophyte community. Seasonal variation has been reported in ^{137}Cs concentrations in emergents (*Carex* and *Equisetum*), with the highest concentrations occurring at the beginning of the growing season (Carlsson and Lidén 1978). The increase in radioactivity in the roots of *E. septangulare – L. dortmanna* in our study suggests that radionuclide translocation to roots may have occurred or surface binding to roots occurred as a result of radionuclide mixing in sediments over time. Redistribution of ^{137}Cs to roots has been reported in *Equisetum fluviatile* (Carlsson and Lidén 1978).

There was little difference in the amount of radioactivity observed in the various minnow species (Fig. 7). This is probably a reflection of the large overlap in the diets and habitat of these species. The diets of these forage fish consist of benthic invertebrates, detritus, aquatic plants, and zooplankton (Tallman et al. 1984). Lower concentrations in pearl dace than in the other species may be partially the result of our selection of larger (~8 cm in fork length) mature specimens for analysis. Mature pearl dace are larger than the other species and live for 3 or 4 years, but most of their growth occurs the first year (Tallman et al. 1984). Therefore, radionuclides taken up by mature pearl dace would be diluted into a larger biomass than would be the case for the other species.

In our study, ^{134}Cs concentrations in forage fish still continued to increase 1 year following the radionuclide addition to the lake (Fig. 7). This trend differed from that when the radionuclides were added to the epilimnion of L266N. Addition of the radionuclides to the epilimnion resulted in maximum ^{60}Co concentrations of 0.07, 0.11, and 0.01 Bq·g^{-1} and ^{134}Cs concentrations of 0.9, 3.2, and 1.0 Bq·g^{-1} in pearl dace, fathead minnow, and slimy sculpin on day 93, or earlier (R. Hesslein, Department of Fisheries and Oceans, Winnipeg, Man., personal communication). These values are lower than the maximum ^{60}Co and ^{134}Cs concentrations of 0.5 and 9 Bq·g dw^{-1} recorded in our study (Fig. 7), despite similar quantities of radionuclides added to the lake. This trend also differed from that observed by Harrison et al. (1990), who added ^{59}Fe, ^{60}Co, ^{65}Zn, ^{75}Se, ^{134}Cs, and ^{203}Hg to the epilimnion of nearby oligotrophic Lake 224 (L224). They reported that concentrations of each isotope rose rapidly, reached a peak at about 100 days, and then declined rapidly in fathead minnow (Harrison et al. 1990). A peak ^{134}Cs concentration of about 1.2 Bq·g dw^{-1} (assuming dry weight is 25% of wet weight) in fathead minnow in their study is also lower than the value recorded in our study, despite similar initial lake water concentrations in the two studies. Assuming instantaneous mixing throughout the lakes, initial ^{134}Cs concentrations in L224 and L226 would have been 4.3 and 4.8 Bq·L^{-1}, respectively. Lower

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Table 4. Summary of bioaccumulation factors (L·kg dw^{-1}) for Co and Cs in freshwater biota from the literature.

Biota	Co	Cs
Invertebrates		
Clams (*Elliptio* sp.)	3 300[1]	
Chironomus luridus	300[2]	
Tadpoles	2 500[1]	
Detritivorous insects		600 – 110 000[3]
Benthic invertebrates		8 300 and 33 700[4]
Amphipods		37 000[5]
Zooplankton		3 500[5]
Phytoplankton		
Algae		1 000 – 500 000[3,4,6,7]
Scenedesmus	15 000[8]	
Macrophytes		
Macrophytes		1 300 – 62 000[3,4,9]
Elodea	40 000[10]	
Equisetum		11 000[11,12]
Yellow water lily		11 000[11,12]
Fish		
Minnows	90–190[1]	
Fathead minnow	950[13]	15 850[13]
Minnow (*Phoxinus phoxinus*)		12 000[14]
Planktivorous fish	3 000[4]	2 570 – 20 500[4]
Omnivorous fish	1 450–3 250[4]	625 – 30 000[3,4]
Benthic omnivorous fish	1 150–1 925[4]	
Carp	11[10]	
Brown trout		3.5[15]
Trout		195 000[16]
Upper Great Lakes		10 000[14]

Note: Dry weight is assumed to be 5% of wet weight for phytoplankton, 10% for invertebrates and macrophytes, and 20% for fish. References (superscripts): 1, Ophel and Fraser (1971); 2, Baudin and Nucho (1992); 3, Reichle et al. (1970); 4, Baylock (1982); 5, Hakonson and Whicker (1975); 6, Williams and Swanson (1958); 7, Lindner et al. (1989); 8, Nucho and Baudin (1986); 9, Trapeznikov et al. (1993); 10, Amiard-Triquet (1979); 11, Kulikov (1973); 12, Carlsson and Lidén (1978); 13, Harrison et al. (1990); 14, Rowan and Rassmussen (1994); 15, Hewlett and Jefferies (1978); 16, Preston et al. (1967).

concentrations in forage fish measured in L226N when the radionuclides were added to the epilimnion than in our study may have been because of growth dilution of the radionuclides as a result of faster growth rates while nutrients were experimentally added to the lake. Also, the radionuclides may have been less available to fish because of a faster rate of loss from water to sediment or faster uptake by algae receiving nutrient enrichment. However, it is not clear why concentrations were lower in forage fish in oligotrophic L224 than in our study.

Uptake rates, k (per day), for Cs by fish are presented in Table 3. Values for forage fish are consistent with those values measured when the radionuclides were added to the epilimnion of the same lake. A higher rate of ^{134}Cs uptake was calculated in L224 ($k = 0.017$ day^{-1}) than in L226N following epilimnion ($k = 0.011$ day^{-1}) or hypolimnion ($k = 0.009$ day^{-1}) addition, although these rates were not different when uncertainty is taken into consideration (Table 3). Uptake coefficients ranged from 0.006 to 0.103 day^{-1} in European lakes following the Chernobyl accident (Table 3). Therefore, uptake rates for Cs by fish are in the low range of those calculated for European lakes. Considering that the uptake rates were consistent among the ELA studies, higher concentrations recorded in our study are probably a reflection of the slower loss rate for ^{134}Cs from water. Loss rate constants from water, corrected for epilimnion deepening, are available for ^{134}Cs when added to the epilimnion of L226N and the epilimnion of L224 (Hesslein 1987) and the hypolimnion of L226N (Bird et al. 1995). On the basis of these loss rate constants, the half-time for ^{134}Cs is 16.5 days in the fertilized epilimnion of L226N, 43 days in the epilimnion of L224, and 99 days in L226N (the whole lake) following lake turnover in autumn.

Most of the radioactivity in the forage fish was from ^{134}Cs. In our study, ^{134}Cs did not biomagnify in the food chain, which is consistent with the finding of others (Reichle et al. 1970; Kolehmainen 1972; Thomann 1981), although biomagnification of ^{134}Cs may occur (Eyman and Kevern 1975; Hammar et al. 1991). Low levels of ^{60}Co in forage fish probably reflect the strong affinity of ^{60}Co to bind to particles and its association with bottom sediments, an observation also made by Klaverkamp et al. (1983). They may also be a reflection of the fact that ^{60}Co concentrations tend to decrease in higher trophic levels (Ophel and Fraser 1971; Baudin and Fritsch 1987). Slightly higher levels of radioactivity occurred in slimy sculpin during the winter months than in minnow species, which may reflect the benthic feeding behaviour of slimy sculpin. Higher ^{60}Co concentrations have been reported in young yellow perch (*Perca flavescens*) that feed on zooplankton than in omnivorous minnows (Ophel and Fraser 1971).

Bioaccumulation factors

BAFs varied considerably over the duration of the study and with taxon. In general, BAF values increased with time as concentrations in water decreased and, in certain cases, as concentrations increased in biota. The single spike addition to the lake results in decreasing concentrations in the water with time as the radionuclides are lost to sediments. In comparison, continuous addition eventually leads to equilibrium conditions. The effect of changing water concentrations and the influence of retention of radionuclides accumulated in biota early in the experiment when water concentrations are elevated are averaged out by expressing our BAFs values as GM values. These values are of use to the nuclear industry in assessing the impact of both chronic and acute releases of radionuclides on the environment.

In general, BAFs measured in our study (Table 1) are consistent with those reported in other studies (Table 4). For example, the BAFs for ^{60}Co in clams ranged from 130 to 780 ww (1450–8700 dw), which is consistent with the BAF of 330 ww for ^{60}Co in clams (*Elliptio* sp.) in Perch Lake, Ontario (Ophel and Fraser 1971). BAFs for invertebrates in our study varied considerably with taxon; the lowest values were found in *Chaoborus* and the highest values in tadpoles. BAFs in periphyton were consistent with or higher than those reported in other studies (Table 4). BAFs for nonroot tissues of macrophyte species varied depending on the species and were in the range reported by others (Table 4). BAFs in forage fish ranged from 130 to 450 ww (534–1800 dw) for ^{60}Co and from 20 to 3000 ww (90 – 12 000 dw) for ^{134}Cs. These values are

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consistent with those for fathead minnow of 190 ww for ^{60}Co and 3170 ww for ^{134}Cs in nearby L224 (Harrison et al. 1990) and are in the range of values reported by others (Table 4).

In conclusion, radionuclides that may enter the bottom water of a lake as a result of nuclear fuel waste disposal will be available to biota and may result in higher concentrations in forage fish than when added to the epilimnion.

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The TRANSAQUA model applied to zebra mussels (*Dreissena polymorpha*) used for the biomonitoring of ^{137}Cs and ^{106}Ru contamination

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Abstract: The dynamics of contamination of the zebra mussel (*Dreissena polymorpha*) by two artificial radionuclides, ^{106}Ru and ^{137}Cs, were quantified in a simplified experimental ecosystem. Main sources of contamination (water, phytoplankton, and other natural suspended matter) were considered. The kinetic constants of accumulation and elimination governing each transfer in the ecosystem were used to calibrate the TRANSAQUA model (TRANSfer of radionuclides along AQUAtic food chains). This model was run to simulate the time series of pollutant concentrations within a population of zebra mussels in the Rhone River downstream of the Marcoule fuel reprocessing plant. The ecological parameters considered included the seasonal cycle of phytoplankton and the mussel clearance rate. The TRANSAQUA model was validated on the basis of a comparison between the simulated results and the data from quarterly γ spectrometry measurements of samples from the mussel population. Almost all field data were within the range of simulated concentrations, except a few points sampled just after periods of flood or low water. For ^{106}Ru, the water pathway was predominant for the whole mussel, almost reaching 80%. For ^{137}Cs, the ingestion of natural suspended matter, other than phytoplankton, was the main route explaining the contamination of the entire mussel.

Résumé : Dans un écosystème expérimental simplifié, nous avons quantifié la dynamique de la contamination de la moule zébrée (*Dreissena polymorpha*) par deux radionucléides, soit le ^{106}Ru et le ^{137}Cs. Notre attention a porté sur les principales voies de contamination (eau, phytoplancton et autres matières naturelles en suspension). Nous nous sommes servi des constantes cinétiques de l'accumulation et de l'élimination qui régissent les phénomènes de transfert survenant dans l'écosystème pour calibrer le modèle TRANSAQUA (TRANSfert de radionucléides le long les chaînes trophiques AQUAtiques). Avec ce modèle, nous avons simulé des séries chronologiques de concentrations de polluants dans une population de moules zébrées du Rhône, en aval de l'usine de retraitement de Marcoule. Entre autres paramètres écologiques, nous avons considéré le cycle saisonnier du phytoplancton et le coefficient d'épuration de la moule zébrée. Pour valider le modèle TRANSAQUA, nous avons comparé les résultats de la simulation aux valeurs obtenues tous les trois mois par spectrométrie γ d'échantillons de la population de moules. Presque toutes les valeurs réelles étaient comprises dans la plage des concentrations simulées, sauf quelques unes résultant d'un échantillonnage après une période de crue ou d'étiage. La contamination de la moule entière par le ^{106}Ru est principalement due à l'eau; elle a presque atteint 80 % par cette voie. Dans le cas du ^{137}Cs, l'ingestion de matières naturelles en suspension autres que le phytoplancton est la principale voie de contamination de la moule entière.
[Traduit par la Rédaction]

Introduction

The zebra mussel (*Dreissena polymorpha*) is a sessile bivalve, common in European waterways. It is of increasing ecotoxicological interest because of its potential as a chemical micropollution bioindicator. The selection criteria for bivalves as pollution bioindicators have been discussed by Phillips (1977) and Bias and Karbe (1985). Various research programmes proposed this mollusc as a sentinel organism for the presence of various contaminants within aquatic ecosystems. A number of studies have dealt with trace metals (Kraak et al. 1991, 1992; Busch et al. 1992; Mersch and Pihan 1993) and others with PCBs or PAHs (Mersch et al. 1992; Bruner et al. 1994*a*, 1994*b*). However, in the field of radioecology, there have been few experimental or in situ studies on the zebra mussel. Yet, whenever populations located downstream of nuclear facilities have been sampled appropriately, most of the radionuclides discharged during normal operation have been detected (Mersch et al. 1992; Kinney et al. 1994). In general, despite the improvements in analytical techniques achieved over the past few years, there are still major methodological difficulties in measuring radionuclides in water and biota because of their low concentrations (Borcherding 1992). The cost and practical difficulties of continuous monitoring are prohibitive, and therefore, this operation is only done occasionally, providing data for only specific moments. Furthermore, water analyses do not provide any information on bioavailability of radionuclides. *Dreissena polymorpha* is relatively insensitive to pollution and exhibits a poor capacity for regulation of metals

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compared with vertebrates, such as fishes (Metcalfe-Smith et al. 1996). This physiological characteristic results in high concentrations in the tissues in relation to the level of exposure or concentration of the pollutant in the water. However, the level of contamination in organisms is influenced by both abiotic and biotic factors, such as size, sex, physiological state, and growth (Jantz and Neumann 1992).

Here, we report a first attempt to model the response of zebra mussels to radioactive contamination, with a view to determining the main factors that govern kinetics. Three major sources of contamination can be identified: filtration of the contaminated water, ingestion of phytoplankton, and ingestion of other natural suspended particles (e.g., mineral and organic particles, bacterioplankton). An examination of the literature reveals that the majority of studies concerning *Dreissena* have been on two radionuclides, ^{106}Ru and ^{137}Cs. These two radionuclides are radioecologically important because they occur in the liquid effluents from fuel reprocessing plants (Bidard and Bardin 1987) and in the atmospheric fallout from the Chernobyl accident (Foulquier and Baudin-Jaulent 1990). On the basis of available experimental data, we applied a standardised procedure for estimating the accumulation and elimination rates of a specific radionuclide, its contamination route, and the receptor organism. These rates were used to calibrate the TRANSAQUA model (TRANSfer of radionuclides through AQUAtic food chains) (Garnier-Laplace 1991; Zhelezniak et al. 1995), which simulated the contamination of a population of mussels in the Rhone River, downstream of the Marcoule fuel reprocessing plant. The γ radioactivity of this population has been monitored for the last 14 years by the ionising radiation protection department of the Marcoule facility.

The TRANSAQUA model is described and the degree of representation of the natural ecosystem by the model is discussed. Comparison of measured concentrations within the mussel samples located in the Rhone River downstream of the Marcoule facility with concentrations simulated by TRANSAQUA illustrates the need for complementary laboratory studies, mathematical modelling, and in situ investigations. A preliminary analysis of the sensitivity of the model was also carried out for the major factors taken into account (river flow rate, algal biomass, suspended matter load, mussel clearance rate).

Material and methods

General description of the laboratory experiments and the mathematical processing of experimental data

Each transfer of radionuclide from one compartment to another was studied individually by examining the physical and chemical quality of the water and the pollutant and the physiological condition, biomass, feeding rate, and food of the zebra mussel. The choice of experimental ecosystem compartments was determined by the Rhone River ecosystem downstream of the Marcoule facility which the TRANSAQUA model was designed to model.

The data concerning the ^{106}Ru and the ^{137}Cs exchanges between water and natural suspended matter were derived from the work of Ferrer (1983). This author studied raw Rhone water collected downstream from the Marcoule facility at key stages in the river's hydrological cycle (low water flow, median flow, and flood), each state being defined by a given concentration of natural suspended matter (from 20 to 140 mg dry weight (dw)·L^{-1}). Three characteristic states were defined on the basis of an analysis of the classified flow curves given by Picat and Debeuns (1972) for the St-Etienne-des-Sorts station (Fig. 1) as follows: low water flow state (flow rate <530 m^3·s^{-1} occurring 10 days·year^{-1}), median-flow state with flow rate between 530 and 3800 m^3·s^{-1} occurring 345 days·year^{-1} (median value equal to 1300 m^3·s^{-1}), and flood state (flow rate >3800 m^3·s^{-1} occurring 10 days·year^{-1}). To represent the exchange kinetics of a radionuclide between the dissolved phase (filtered water at 0.45 µm with a concentration of $C_w(t)$) and the particulate phase (natural suspended matter with a concentration of $C_{sm}(t)$), a very simple model based on the first-order kinetics such as $dC_w/dt = -\alpha C_w$ was selected. Given the initial conditions defined by $C_w(0) = C_w^0$ and $C_{sm}(0) = C_{sm}^0$, the fraction $F(t)$ that has disappeared from the dissolved phase after a contact time t can be written

$$(1) \qquad F(t) = 1 - \frac{C_w^0}{C_w^0 + C_{sm}^0} e^{-\alpha t} = 1 - \beta e^{-\alpha t}$$

where $F(t)$ can be assimilated to the fraction fixed by the suspended matter. The parameters for the model (α, β) were estimated by linear regression on the basis of the experimental data series (t, $F(t)$) of Ferrer (1983).

The laboratory protocols used to quantify the transfer to the planktonic green alga and the zebra mussel have been described by Vray (1994) and Sombré (1987), respectively, for ^{106}Ru and ^{137}Cs. The general design of these experiments was similar, allowing the same mathematical processing of acquired data. Here, we offer a brief description of the data processing for estimating the accumulation and depuration kinetics of each transfer studied. For each transfer, the experiment was divided into two phases for which the radioactivity of the source of contamination was accurately known and that of the two main tissues of the receptor organism (mussel shells and soft tissues) was monitored. During the uptake phase, the receptor organism accumulated via an isolated source of contamination, such as water, natural suspended matter, or phytoplankton, and simultaneously eliminated the radionuclide. The contaminated receptor organism was then placed in a non-radioactive environment to study depuration.

The processing of the experimental data was based on a compartmental model in which the receptor organism (or tissues) and its environment were represented by a group of compartments between which the exchange occurred. The basic assumptions and the initial conditions required to solve the first-order differential system that comprises the compartmental model and the corresponding analytical solutions have been described by Garnier-Laplace et al. (1997) and are not given here. The radionuclide transfer kinetics parameters u_i for uptake rate (per day) and d_i for depuration rate (per day) appear in all the analytical solutions. They were estimated by means of regressions from experimental data, taking into account radionuclide concentration fluctuations in the contamination source. The experiment was divided into appropriate time intervals in which the radionuclide concentration in the source of contamination could be described by a constant (Le Fur 1989; Vray 1994). To ensure easy reading, the main equations are presented after correction of all concentrations by the physical decay of the radionuclide (372 days and 30 years for ^{106}Ru and ^{137}Cs, respectively) and the growth of the receptor organism. By considering n time intervals j from time 0 to time T_{j+1} ($j \in$ N), the concentration of the radionuclide in the source of contamination $C_s(t)$ is given by

$$(2) \qquad C_s(t) = C_{s_{j+1}}, \ \forall t \in \ ]T_j, T_{j+1}]$$

The concentration within the receptor organism (or tissues) $C_r(t)$ schematised by m compartments is then written

$$(3) \qquad C_r(t) = \sum_{i=1}^{m} \frac{u_i}{d_i} \left[\sum_{j=1}^{n} C_{s_j} \left(e^{-d_i(t-T_j)} - e^{-d_i(t-T_{j-1})}\right) + C_{s_{j+1}} \left(1 - e^{-d_i(t-T_j)}\right) \right]$$

$$\forall t \in \ ]T_j, T_{j+1}]$$

Let

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$$D_{i,j+1} = \frac{C_{s_j}}{d_i}(e^{d_i T_j} - e^{d_i T_{j-1}}) + D_{i,j}, \text{ with } D_{i,1} = 0$$

The expression simplifies to

$$(4) \quad C_r(t) = \sum_{i=1}^{m} u_i \left[e^{-d_i t} D_{i,j+1} + \frac{C_{s_{j+1}}}{d_i}(1 - e^{-d_i(t-T_j)}) \right]$$

Knowing the values C_{s_j} and d_i, it is possible to calculate a variable $X_i(t)$ for each compartment i and for each time step, defined by the formula

$$(5) \quad X_i(t) = e^{-d_i t} D_{i,j+1} + \frac{C_{s_{j+1}}}{d_i}(1 - e^{-d_i(t-T_j)})$$

$$\forall t \in]T_j, T_{j+1}], \text{ with } D_{i,1} = 0$$

The u_i parameter values were thus estimated by a multiple linear regression without a constant term as $C_r(t)$ is written

$$(6) \quad C_r(t) = \sum_{i=1}^{m} u_i X_i(t).$$

Field data from the biomonitoring of an autochtonous Rhone population

Sampling sites for mussels and water are shown in Fig. 1. The organisms were sampled quarterly as part of the aquatic environment monitoring programme carried out in the vicinity of the Marcoule fuel reprocessing plant. This monitoring programme was undertaken by the facility's radiation protection department. Each sample contained about 2 kg wet weight (ww) of specimens of all sizes taken from the Avignon dam by means of a rake. The mussels collected were washed with running water, dried at 65°C for 3 days, ground, and then ashed at 730°C for a 12-h period, following a temperature rise in stages over 6 h. The radioactivity of the ash was measured using low-level γ spectrometry. The concentrations recorded were expressed relative to fresh weight. The confidence interval at 95% relative to measurement error represented an average of 31 and 37% of the concentrations recorded, respectively, for ^{106}Ru and ^{137}Cs.

The radionuclide concentration in the water was measured by means of γ spectrometry based on 30-L samples collected on a monthly basis and comprising aliquots gathered daily by a hydrocollector once the decantation process had been carried out. The water sampling facility was located at Roquemaure. Each sample was concentrated prior to measurement by elution through an ion-exchange resin. The column was 3.5 cm in diameter and 5.2 cm in length and contained 280 g of mixed resin (40% of C20 cationic resin and 60% of A101 anionic resin) with a flow rate of 624 mL·h^{-1}. The resin was then dried in the open air for a 24-h period before measurement. The detection limit, under these operating conditions, was close to 9.25×10^{-2} Bq·L^{-1} for ^{106}Ru and 1.85×10^{-2} Bq·L^{-1} for ^{137}Cs. The confidence intervals associated with the measured concentrations linked to the measurement errors were equal to 49% for both radionuclides.

Description of the model used

The TRANSAQUA model simulates the response of a receptor organism at a given trophic level, in this case the zebra mussel, a first-order consumer, on the assumption that the different transfer pathways were additive. This model includes various submodels as a function of the hydroecological factors that must be taken into account to simulate the contamination by a given pollutant of a given ecosystem. It has already been tested for various fish species from certain lakes and reservoirs (Garnier-Laplace 1991; Zhelezniak et al. 1995). Its adaptation to a river ecosystem was initiated by Vray (1994).

Hydrological submodel

For simulation of the contamination of the zebra mussels located at the Avignon dam station (AVI), 28.5 km downstream from the discharge point, the available data for radionuclide concentrations in the water were monthly records basis at the Roquemaure station (ROQ), 11.5 km downstream of the Marcoule plant. The concentration of radionuclides in water C_w^{AVI} and in natural suspended matter C_{sm}^{AVI} at the Avignon dam were calculated by means of the simplified hypothesis for the maintenance of the quantity of radionuclides between the two stations and the maintenance of the flow rate (Q) and the suspended matter load (SML) for the studied section. The main purpose of the TRANSAQUA model is the simulation of contamination in biological compartments. Transport of solids (erosion and deposit/suspension) were not taken into account. Taking these phenomena into account would necessitate a large-scale field study to calibrate the submodels used. The equations used for each time interval j, for a duration corresponding to the acquisition frequency for the radionuclide concentrations in the water (monthly frequency), were as follows:

$$(7) \quad C_{w\,j}^{AVI} = C_{w\,j}^{ROQ} \frac{F_j^{ROQ}(\Gamma_j^{ROQ})}{F_j^{AVI}(\Gamma_j^{AVI})}$$

and

$$(8) \quad C_{sm\,j}^{AVI} = C_{w\,j}^{AVI} \frac{F_j^{AVI}(\Gamma_j^{AVI})}{1 - F_j^{AVI}(\Gamma_j^{AVI})} \frac{1}{SML_j}$$

with Γ_j^{ROQ} and Γ_j^{AVI} the time (days) required for a drifting particle to cover, at an average flow speed, the distance between the effluent discharge point and the station shown as an exponent. These variables were calculated from the average isospeed curves for the flow depending on the flow rate ($v(Q)$) given by Picat and Debeuns (1972) for each hydraulically homogenous section from the effluent discharge point to the Avignon dam:

$$(9) \quad \Gamma_j^{AVI} = \frac{d^{AVI}}{v(Q_j)}$$

and

$$(10) \quad \Gamma_j^{ROQ} = \frac{d^{ROQ}}{v(Q_j)}$$

where Q_j is the average monthly flow measured at Roquemaure (cubic metres per second), which was considered to be constant from the discharge point to the Avignon dam, and d^{ROQ} and d^{AVI} are the distances between the discharge point and the station shown as an exponent, 11.5 and 28.5 km, respectively.

The suspended matter load, SML_j (milligrams dry weight per litre), was calculated by means of the relationship obtained by linear regression on a series of 47 pairs (Q, ln(SML)) obtained other than during the flood or low water periods. These occasional data were supplied by the "Agence de l'eau Rhone-Méditerranée-Corse" and complete the measurements from the Avignon station:

$$(11) \quad SML = e^{0.00174Q} \quad (r^2 = 0.96)$$

For flood states, the upper level for the suspended matter concentration has been set at 106 mg dw·L^{-1} (average value for the 11 available pairs). For low-water states, the value has been set at 4 mg dw·L^{-1} (average value for the seven available pairs).

The radionuclide fraction, F_j^{ROQ} and F_j^{AVI} (litres per kilogram), fixed by the suspended matter for a contact time equal to Γ_j^{ROQ} and Γ_j^{AVI}, respectively, was calculated by means of the following equations:

$$(12) \quad F_j^{ROQ} = 1 - \beta(Q_j)\, e^{-\alpha(Q_j)\Gamma_j^{ROQ}}$$

and

$$(13) \quad F_j^{AVI} = 1 - \beta(Q_j)\, e^{-\alpha(Q_j)\Gamma_j^{AVI}}$$

where $\alpha(Q_j)$ and $\beta(Q_j)$ are the parameters for the model estimated by means of the experimental data of Ferrer (1983) on the basis of the stage within the Rhone's hydrological cycle.

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Phytoplankton submodel

The basic hypotheses adopted assume that phytoplankton are particles that drift at the average flow speed and whose cellular division linked to growth can be ignored for an initial maximising simulation, given the rapidity and the intensity of the radionuclide fixation by the cells and the short time between the point of discharge of effluents and the Avignon dam (Γ_j^{AVI} always less than 2 days). The average radionuclide concentration in phytoplankton for time interval j, at the Avignon dam where these organisms are eaten by zebra mussels, is given by

$$(14)\quad C_{p_j}^{w} = C_{w_j}^{AVI}\left[\sum_{i=1}^{m} \frac{u_{p_i}^{w}}{d_{p_i}^{w}+\lambda_p}\left(1-e^{-(d_{p_i}^{w}+\lambda_p)\Gamma_j^{AVI}}\right)\right]$$

where $u_{p_i}^{w}$ and $d_{p_i}^{w}$ (per day) characterise the accumulation and depuration kinetics parameters that quantify the exchange between the source of contamination shown as an exponent (water) and the receptor organism shown as a suffix (phytoplankton) for a given radionuclide and λ_p characterises the radionuclide physical decay (per day). This parameter value is equal to 6.33×10^{-5} and 1.86×10^{-3}·day^{-1}, respectively, for ^{137}Cs and ^{106}Ru.

Zebra mussel submodel

The field data are based on in toto mussel samples of all sizes. To simulate the average concentration representative of the entire population comprising various cohorts, the radionuclide concentration in zebra mussels was calculated for a standard average-sized individual (22 mm in length) that does not grow and whose mass distribution pattern of the various tissues remains identical throughout the season. The choice of these maximising assumptions is a simple way to buffer variations between individuals and between cohorts, linked to differences in the degree of maturity that may occur within a given population consisting of juveniles and adults at varying stages of maturity, including seniles individuals. The only physiological parameter taken into account for each time interval j for the simulation is the clearance rate CR_d (millilitres per hour). This parameter, which is particularly affected by the suspended matter load characterising the ecosystem, varies in accordance with eq. 15, based on the experimental data provided by Lei et al. (1996) obtained for individuals of a standard size (length 22 mm):

$$(15)\quad CR_{d_j} = 193e^{-0.0153SML_j}\ (r^2 = 0.96).$$

The simulation was based on the assumption that radionuclide concentrations for the various sources of contamination were constant per time interval of a month (acquisition time step of the concentration in the water) and were therefore defined in accordance with eq. 2. The contamination of zebra mussels by a given source "s" was calculated in accordance with eq. 4 which was corrected according to the main ecological factor taken into account P_d^s. This correction was applied on the assumption of a linear response for the organism between the factor value $P_{d\,situ_j}^s$, characterising the in situ ecosystem (Avignon dam) for time interval j, and the value $P_{d\,exp}^s$ characteristic of the experiment carried out to quantify the kinetic transfer parameters during which the factor was controlled and maintained at a constant value. This hypothesis is supported by $P_{d\,situ_j}^s$ and $P_{d\,exp}^s$ being always of the same order of magnitude. Equation 4 became for each source of contamination "s" for *Dreissena polymorpha* "d"

$$(16)\quad C_d^s(t) = \sum_{i=1}^{m} u_i\left[e^{-d_i t}D_{i,j+1} + \frac{P_{d\,situ_j}^s}{P_{d\,exp}^s}\frac{C_{s_{j+1}}}{d_i}\left(1-e^{-d_i(t-T_j)}\right)\right]$$

with

$$D_{i,j+1} = \frac{P_{d\,situ_{j+1}}^s}{P_{d\,exp}^s}\frac{C_{s_j}}{d_i}\left(e^{d_i T_j} - e^{d_i T_{j-1}}\right) + D_{i,j},\ \text{with}\ D_{i,1} = 0.$$

In eq. 16 for direct contamination, the source of contamination "s" was water and P_d^w was assimilated to the clearance rate CR_d. For trophic transfer, the factor taken into account was the phytoplankton ingestion rate which was the product of the clearance rate (millilitres per hour) and the algal biomass (AB, milligrams wet weight per litre) (in eq. 16, the source of contamination "s" is phytoplankton and P_d^p was assimilated to the product $CR_d \times AB$). The ecological cycle for this biomass has been modelled by reproducing, for the entire studied period, the 2-year chlorophyll *a* concentrations (milligrams per litre) measured at the Avignon station by the "Agence de l'eau Rhone-Méditerranée-Corse." These concentrations were transformed into milligrams wet weight per litre on the basis of the relationship established by Nucho (1989) for *Scenedesmus* (AB = 6.7 × [chlorophyll *a*], $r^2 = 0.98$). As for the phytoplankton, radionuclide transfer due to the ingestion of the other natural suspended matter was corrected taking into account in eq. 16 the factor P_d^{sm} assimilated to the product $CR_d \times SML$.

For each source of contamination, the concentration in the zebra mussel was calculated for the main tissues involved in the transfer, i.e., soft tissues when dealing with contamination due to ingestion of algae or other natural suspended matter and soft tissues and shells when the source of contamination was water. The concentration for the whole organism $C_d(t)$ was recreated by applying a simple weighting by the mass contribution (wet weight) for the tissues considered (m_{st} and m_s equal to 0.18 and 0.56, respectively, for the soft tissues (st) and the shells (s)) to the in toto mass. Using the transfer pathway additivity, the concentration in the in toto mussels was calculated as follows for time t:

$$(17)\quad C_d(t) = (m_{st}C_{d_{st}}^{w}(t) + m_s C_{d_s}^{w}(t)) + m_{st}C_{d_{st}}^{p}(t) + m_{st}C_{d_{st}}^{sm}(t)$$

To calculate the concentration in mussels at time 0 of the simulation, we assumed that the initial observed value (sample for January 6, 1982) resulted from an equilibrium within the mussel population in the Rhone River in median-flow state. Two simulations were carried out for each radionuclide: one with the minimum radionuclide concentration in the water as input data (zero if the concentration is less than the detection limit, and the measured value minus the confidence interval at 95% otherwise) and the other with the maximum concentration in the water (detection limit value if the concentration is nonmeasurable, and the measured value plus the confidence interval at 95% otherwise).

The accumulation and depuration kinetic parameters used to calibrate equations of the TRANSAQUA model are listed in Table 1.

Results

Global sensitivity analysis

As the hydroecological factors taken into account are interdependent, an initial sensitivity analysis was carried out to study the influence of the three characteristic states in the Rhone River's yearly cycle downstream of the Marcoule plant. For the median-flow state, defined by a natural suspended matter load of about 10 mg dw·L^{-1} and an algal biomass of 40 mg ww·L^{-1} (Table 2), the clearance rate for the mussels and the time taken for a particle to drift between Marcoule effluent discharge point and the Avignon dam were 166 mL·h^{-1} and 0.64 day, respectively. For ^{106}Ru, at equilibrium, this situation would lead to a contribution by water, phytoplankton, and other natural suspended matter of 54, 11, and 35%, respectively, of the total radionuclide concentration in the whole mussel. The phytoplankton source reached a maximum relative contribution of 20% in a low-water period during which the algal biomass was significant and the contact duration prolonged (low average flow speed). The same was true for the natural suspended matter whose contribution could reach 47% under these hydrological conditions. For ^{137}Cs, the

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Table 1. Kinetic parameters estimated on the basis of experimental studies for ^{106}Ru and ^{137}Cs in a simplified ecosystem representative of the lower part of the Rhone River.

		^{106}Ru			^{137}Cs		
Source of contamination	Receptor	Kinetic rates[a]	Experimental parameter[b]	Reference	Kinetic rates[a]	Experimental parameter[b]	Reference
Water	Suspended matter						
	Low flow rate	β: 56 α: 0.56	$SML_{exp} = 20$	Ferrer 1983	β: 75 α: 0.072	$SML_{exp} = 20$	Ferrer 1983
	Medium/high flow rate	β: 76 α: 0.63	$SML_{exp} = 38/140$		β: 68 α: 0.46	$SML_{exp} = 38/140$	
Water	*Scenedesmus*	u_1: 16000 d_1: 18.9	Chronic exposure corrected for growth	Vray 1994	u_1: 1317 d_1: 0.693		Coughtrey and Thorne 1983
Water	*Dreissena* sp. soft tissues	u_1: 4.1 u_2: 1.52×10^{-1} d_1: 3.79×10^{-1} d_2: 8.90×10^{-3}	$CR_{d\,exp} = 193$	Vray 1994	u_1: 30 u_2: 2.67 d_1: 1.29 d_2: 4.30×10^{-2}	$CR_{d\,exp} = 193$	Sombré 1987
	Dreissena sp. shells	u_1: 25.8 u_2: 1.65 d_1: 0.28 d_2: 1.48×10^{-2}			u_1: 50 u_2: 3.31 d_1: 2.81 d_2: 4.80×10^{-2}		
Natural suspended matter	*Dreissena* sp. soft tissues	u_1: 4.33×10^{-3} u_2: 1.99×10^{-5} d_1: 6.24 d_2: 5.17×10^{-2}	$CR_{d\,exp} = 179$ $SML_{exp} = 4.9$	Vray 1994	u_2: 9.55×10^{-5} d_2: 2.62×10^{-3}	$CR_{d\,exp} = 178$ $SML_{exp} = 4.9$	Estimated[c]
Scenedesmus	*Dreissena* sp. soft tissues	u_1: 7.52×10^{-4} u_2: 1.24×10^{-4} d_1: 4.08 d_2: 3.72×10^{-2}	$CR_{d\,exp} = 193$ $AB_{exp} = 82$	Vray 1994	u_1: 1.33×10^{-2} u_2: 3.80×10^{-4} d_1: 1.55 d_2: 8.22×10^{-3}	$CR_{d\,exp} = 193$ $AB_{exp} = 143$	Sombré 1987

[a] α, β, parameters of the model of exchange of the radionuclide between water and natural suspended matter, adapted to the Rhone ecosystem; u_i, uptake kinetic rate per day; d_i, depuration rate per day for compartment i of the organism.

[b] Controlled parameters during the experiment: $CR_{d\,exp}$, clearance rate of *Dreissena polymorpha* ($mL \cdot h^{-1}$); SML_{exp}, suspended matter load (mg ww$\cdot L^{-1}$); AB_{exp}, algal biomass (mg ww$\cdot L^{-1}$).

[c] For this transfer not available in the literature, kinetic rates were estimated to minimize the least-squares criteria calculated for observed concentrations at Avignon station and simulated concentrations for in toto mussels obtained for available transfer data (water and phytoplankton).

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Table 2. Contribution of the different pathways to the radionuclide concentration in the whole mussel for three characteristic hydrological states in the lower part of the Rhone River.

	Equation used	Characteritic condition of flow rate		
		Low	Medium	High
Flow rate, Q ($m^3 \cdot s^{-1}$)	—	530	1300	3800
Natural suspended matter load, SML (mg dw·L^{-1})	11	4	10	106
Contact time Γ (days)	9	1.50	0.64	0.23
Mussel clearance rate, CR_d ($mL \cdot h^{-1}$)	15	181	166	39
Algal biomass, AB (mg ww·L^{-1})	—	120	40	26
Contribution (%)				
Water				
^{106}Ru		33	54	74
^{137}Cs		4	8	7
Phytoplankton				
^{106}Ru		20	11	9
^{137}Cs		57	25	5
Natural suspended matter (other than phytoplankton)				
^{106}Ru		47	35	16
^{137}Cs		39	67	88

Note: Results are of the TRANSAQUA model for equilibrium within the autochtonous mussel population at the Avignon dam station.

phytoplankton contribution is greatest during low-water periods (57% for the entire mussel). The contribution of other natural suspended matter during the flood period was 88%. The contribution of water remained modest, varying between 4 and 8% depending on the hydroecological conditions.

Comparison between simulated and measured concentrations in mussels

We stress that a different predictive weight must be attributed to the simulations for ^{106}Ru which were run as blank and to those for ^{137}Cs for which in situ data were used to estimate two missing kinetics parameters quantifying the transfer between natural suspended matter (other than phytoplankton) and mussel soft tissues.

For better comparison between simulated and measured concentrations, the contamination for the entire mussel was simulated excluding the internal liquids. In fact, it is probable that most of these liquids are lost during transport to the laboratory and during desiccation in the oven. Therefore the contribution of the radioactivity of these liquids to the total concentration in the mussels should not be included in the simulations.

The flow rate of the Rhone River was recorded at the Roquemaure station (Fig. 2) and the fluctuations in the radionuclide concentration in water at the same site followed the hydrological variations (Figs. 3A and 4A). A downward trend for these concentrations was registered, linked to the drop in radioactivity discharged by the Marcoule facility resulting from the opening of the new liquid effluent reprocessing plant in 1993 (Lévy et al. 1993). Overall, for the data series obtained over 14 years of biomonitoring, the model reproduced both the concentration levels and the variations in the whole mussels for both radionuclides (Figs. 3B and 4B). Almost all the points observed are located within the minimum and maximum range of the simulated concentrations. The concentration variations in the whole mussel perfectly match the radionuclide concentration variations in the water. Taking into account the interval of confidence on the measurement, four exceptions should be noted for ^{106}Ru. (*i*) The concentration measured in January 1990 was weaker than the minimum value simulated. This measurement immediately followed a period of low water flow. On the other hand, the other three points, which were greater than the maximum values simulated, resulted from measurements taken after periods of flood: (*ii*) April 1982, (*iii*) January 1983, and (*iv*) April 1988. For ^{137}Cs, two samples (measured concentrations and 95% confidence intervals) resulting from measurements taken after periods of flood were outside the minimum and maximum simulated concentrations: (*i*) the April 1982 and (*ii*) July 1983 samples. The ^{137}Cs model results both validated the entire modelling procedure and provided a basis for estimating the dynamics of the natural suspended matter (other than phytoplankton) pathway.

The simulated concentrations for the soft tissues were far greater than the concentrations in the whole mussel (eq. 17). This underlines the value of separate in situ monitoring of these tissues.

Analytical potential of the model

The analytical potential of the model is such that it can contribute to understanding of the responses of organisms to the variations of concentrations in water. For ^{106}Ru (Fig. 5), most of the time the water pathway was predominant for the whole mussel, almost reaching 80%. This can be explained by the intense contamination of the shells by this source. The concentration level in the whole mussel due to the ingestion of phytoplankton was weak, with the highest values (up to 35%) during low-water periods corresponding to blooms. The other natural suspended matter contamination source played a far more modest role (about 30% on average) than the water except during low-water periods (radionuclide concentration of the suspended matter is greater, in correlation with the longer transit time and the strong clearance rate). For ^{137}Cs (Fig. 6), the contribution of the water pathway to the contamination of the entire mussel or the soft tissue remained modest (about 10%). During flood periods, it occasionally reached 30%. The phytoplankton contributed to the overall contamination of the mussel at a rate of the same order of magnitude (10%), with a marked increase during low-water periods (up to 30%). The other natural suspended matter sometimes contributed over 80% of the overall concentration for the zebra mussel, with maximum values recorded during periods of flood. In contrast with ^{106}Ru, the floods contributed to a slight increase in the contribution from the natural suspended matter, due to the significant reduction from phytoplankton (Table 2).

Discussion

Fidelity of the experimental model to the natural ecosystem

The natural suspended materials are a source of contamination that cannot be discounted for the molluscs which filter by filling the digestive system and (or) indirectly by desorption of

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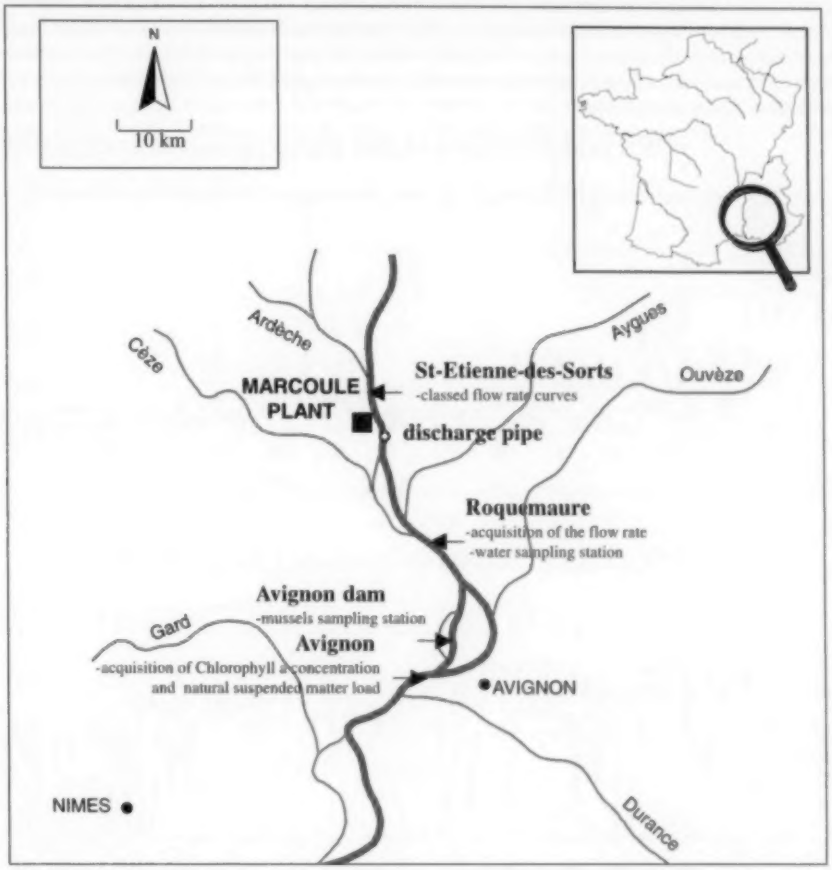
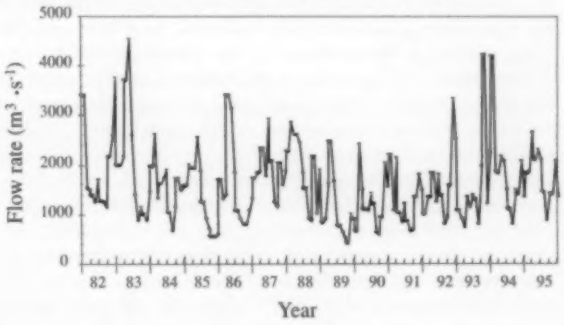
Fig. 1. Location of the sampling stations downstream of the fuel reprocessing plant at Marcoule (Rhone River, France).



the pollutant followed by assimilation. This highly complex source of contamination has been greatly simplified. Only the phytoplankton has been distinguished from all other constituents which include mineral matter, organic matter, and bacterioplankton. This simplification may be justified because phytoplankton is one of the major trophic vectors for the zebra mussel, whose retention efficiency is maximum for algal cells of 5–35 µm (Sprung and Rose 1988). Nevertheless, the bacterioplankton certainly play a trophic role for zebra mussels that is still poorly documented in the literature. Using the experimental approach described here, the variation in time of the physical, chemical, and biological composition of the suspended matter, excluding phytoplankton, is taken into account by the use of a single model to quantify the exchanges between solid and dissolved phases (eq. 1).

Fig. 2. Temporal variation of the Rhone River flow rate at the Roquemaure station. Values represent monthly averages.



Fidelity of the hydrological submodel to the natural ecosystem

Some concentrations recorded in zebra mussels associated with their interval of confidence at 95% on the measurement were situated outside the range simulated. Almost all these points, except one for ^{106}Ru, resulted from samples taken following a flood period. To improve the simulated response of mussels, we need to take into account the particular nature of the watershed governing the hydraulic regime and thus the quality of natural suspended matter which is specific to each flood. As regards the low water flow states for which the measurement of a single sample was outside the simulated range (the July 1983 sample for ^{106}Ru), the limited effect on the natural environment as a result of the duration of the phytoplanktonic blooms in the European waterways of about

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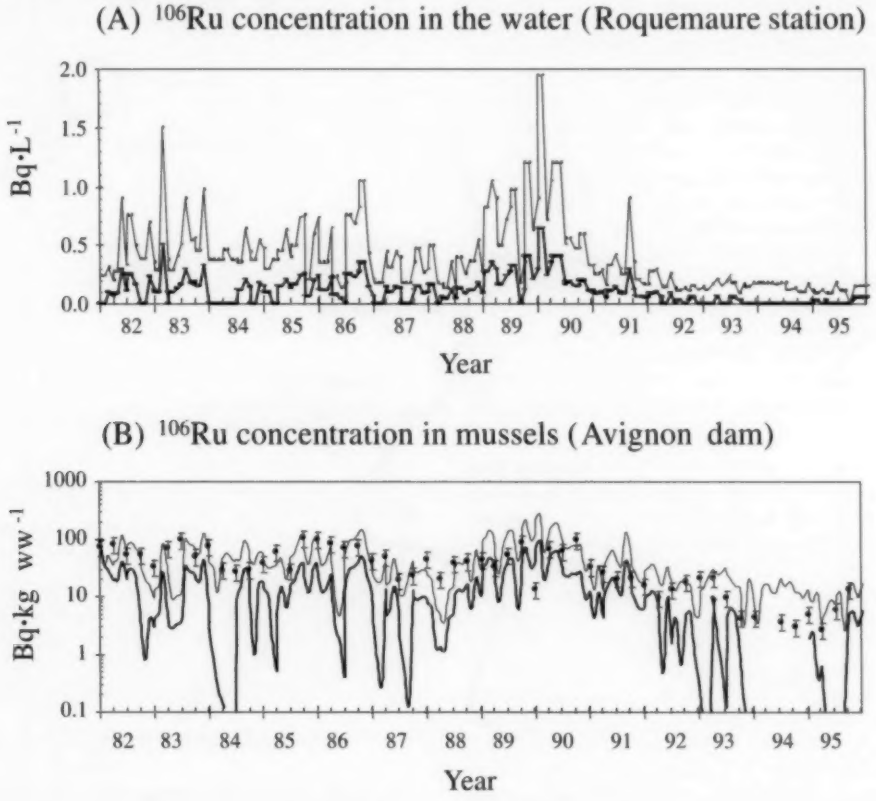
Fig. 3. (A) Model input data for ^{106}Ru. Thin line, maximum values, i.e., detection limit for non-measurable concentrations and upper boundary of the interval of confidence for measured concentrations; bold line, minimum values, i.e., 0 for non-measurable concentrations and lower boundary of the interval of confidence for measured concentrations. (B) Observed and simulated minimum and maximum concentrations for the radionuclide in the in toto mussels. Simulations were run as blank, without using the in situ data series. Circles, measured concentrations in whole mussels with 95% confidence intervals; thin line, simulation with maximum values for the concentration in water; bold line, simulation with minimum values for the concentration in water.



30 days and the limited contact time resulting from the drift of the planktonic particles should be noted. In a lotic environment, notably in the lower part of the Rhone and for the ecosystem studied (Avignon dam), the influence of phytoplankton as a source of contamination proved to be limited as a result of the contamination levels related to the contact time, which at all flow rates remained less than 2 days. In the event of a flood characterised by a high suspended matter load, the radionuclide concentration in the animal resulting from this source of contamination does not normally increase dramatically because the clearance rate for the zebra mussel is reduced by the more or less complete closure of the valves as a protective mechanism (Janssen et al. 1992). Moreover, the radionuclide concentration in the solid particles is lower than during low or median flows as a result of the short contact time and high suspended matter load (dilution among many particles). The hydrology of the river has been simplified by the integration of the phenomena over 1 month, thus smoothing variations occurring over this period. The simulation of zebra mussel contamination using this type of data can only show an overall pattern for the population. Modelling of the solid transport in an area containing two dams is required to improve the accuracy and realism of the relationship between the liquid and solid fluxes. This relationship is often complex.

Improving the model's explanatory potential by adaptation of the field sampling design

To improve the model's explanatory potential and thereby our understanding of the contamination mechanism for the zebra mussel in relation to variations in the hydroecological factors for the waterway, it is necessary to adapt the field sampling and data processing protocols. In fact, excluding the palleal liquids from the simulation results in a bias. For ^{106}Ru, Vray (1994) concluded that after a 90-day transfer from the water, the contribution to the total radioactivity for the organism resulting from this liquid was greater than 25%. The liquid was likely to contain contaminated feces, pseudofeces, and a fraction of the radionuclide which could have been desorbed from the soft tissues. Sombré (1987) demonstrated a 15% contribution resulting from the palleal liquid after a 7-day period of direct ^{137}Cs contamination. The existence of this compartment raises a problem when comparing experimental and field data by introducing a random character linked to the sampling procedures, which could, for example, explain recorded values

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Fig. 4. (A) Model input data for ^{137}Cs. Thin line, maximum values, i.e., detection limit for non-measurable concentrations and upper boundary of the interval of confidence for measured concentrations; bold line, minimum values, i.e., 0 for non-measurable concentrations and lower boundary of the interval of confidence for measured concentrations. (B) Observed and simulated minimum and maximum concentrations for the radionuclide in the in toto mussels. Simulations were run after calibration of the kinetic parameters quantifying the pathway "natural suspended matter → mussel soft tissues" on the basis of minimisation of the least-squares criteria calculated for observed concentrations at Avignon dam station and simulated ones for whole mussels obtained for available transfer data (water and phytoplankton). Circles, measured concentrations in whole mussels with 95% confidence intervals; thin line, simulation with maximum values for the concentration in water; bold line, simulation with minimum values for the concentration in water.

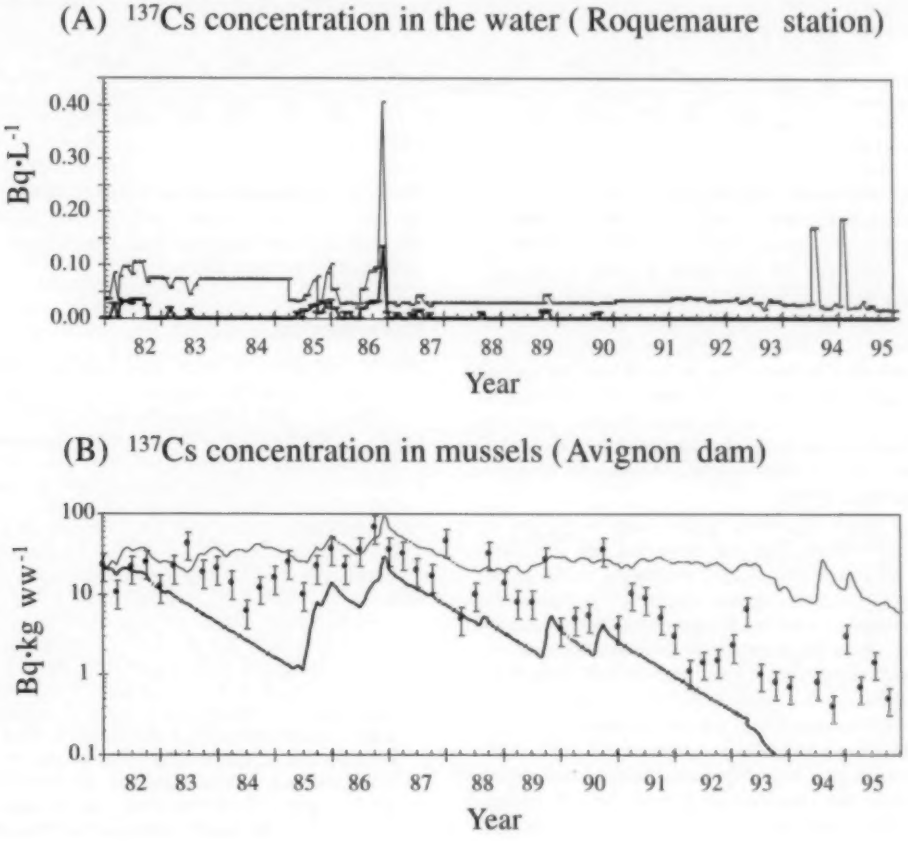

Fig. 5. Temporal variation of the relative contributions for each source of contamination to the simulated ^{106}Ru total concentration in the whole mussels. Thin line, water; bold line, other natural suspended matter; bold line with dashes, phytoplankton.

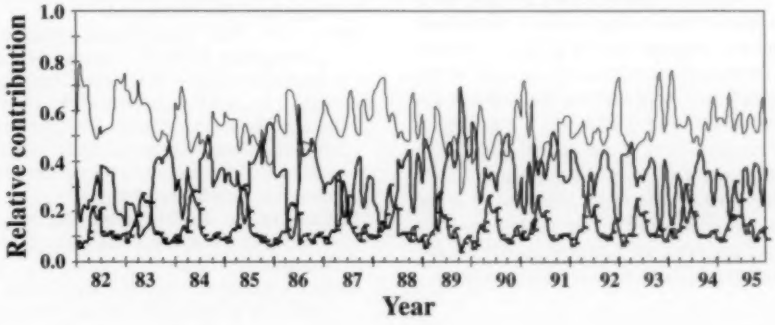

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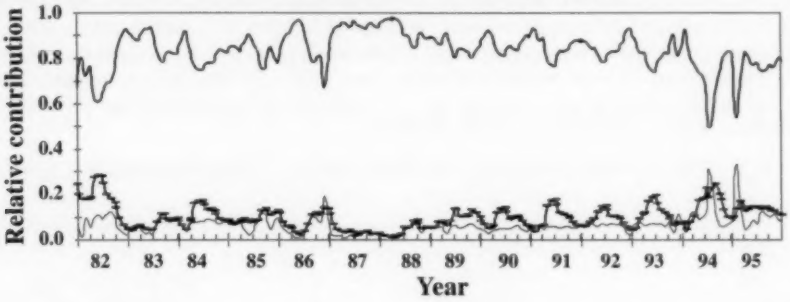
Fig. 6. Temporal variation of the relative contributions for each source of contamination to the simulated ^{137}Cs total concentration in the whole mussels. Thin line, water; bold line, other natural suspended matter; bold line with dashes, phytoplankton.



outside the minimum–maximum range for simulated concentrations. To eliminate this problem, it would be preferable to work with radionuclide concentrations for the soft tissues in the field data rather than for the whole mussel. These data would be of further interest in that these tissues are likely to be consumed by organisms of the upper trophic levels within the ecosystem (Bruner et al. 1994*b*). Furthermore, given that the fixation of the pollutant in the various parts of the zebra mussel is governed by mechanisms that are assumed to be different, it is important to make a distinction for these tissues. A number of authors consider that only the soft tissues enable a correlation between the concentration and its environment to be established (Busch et al. 1992).

Monitoring a single size-class would make it possible to improve the comparisons between measured and modelled concentrations by enhancing their explanatory potential. In this context, one of the most important factors to be taken into account is the pattern of seasonal fluctuation of the mass contribution of each tissue which probably follows the reproductive cycle (Borcherding 1992; Smit and Dudok Van Heel 1992). Smit and Dudok Van Heel (1992) monitored fluctuations in the biomass of mussels of 10 mm in length and demonstrated that the seasonal variations accounted for 73% of the variance observed over a year. The same authors underlined the fact that during the reproductive period, the soft tissues could increase their biomass by up to 75% (ash-free dry weight) compared with the sexual rest period. The mass contribution of these tissues to the overall biomass (wet weight) for a standard-sized mussel therefore increased from 18 to 32%, affecting the pollutant concentration in the whole mussel.

Overall, this study shows the importance in carrying out a standardised approach with regard to the in toto bioindicator organism to estimate the accumulation and depuration kinetics for the radionuclides. To negate the effects of size and of the seasonal weight variations, it is necessary to standardise the use of *Dreissena* as radionuclide bioindicator, for example, with a specific size-class and for a given period within the reproductive cycle. The impact of occasional occurrence of population stress by different factors (e.g., presence of other pollutants or lack of oxygen) should be also evaluated. This approach is essential for planning of future research on the transfer processes of radionuclides to freshwater organisms.

Acknowledgements

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Phylogeography and postglacial dispersal of lake trout (*Salvelinus namaycush*) in North America

Christopher C. Wilson and Paul D.N. Hebert

Abstract: We used restriction fragment length polymorphism (RFLP) analysis of mitochondrial DNA (mtDNA) diversity to assess the complex postglacial history of lake trout (*Salvelinus namaycush*) and test existing dispersal hypotheses. A pilot survey with 30 restriction enzymes was carried out on lake trout from 16 geographically representative populations to determine phylogenetically informative characters. Subsequent screening of 1416 lake trout from 93 populations across the species' range with nine variable restriction enzymes showed that lake trout from at least five glacial refugia contributed to extant populations. Three major mtDNA lineages were observed, with sufficient differences to suggest their divergence during the mid-Pleistocene. Geographic and genetic differences within two lineages suggested further vicariant divergence caused by Wisconsinan glacial advances. In contrast with more southern freshwater species, no correlation was observed between the geographic proximity of glacial refugia and relatedness of mtDNA lineages. Current distributions of refugial lineages are readily explained by consideration of timing and connections of proglacial lakes. These lakes facilitated large-scale dispersal from multiple refugia, particularly enabling long-distance dispersal from the Mississippian and northwestern refugia. Proglacial lakes also enabled extensive secondary contact among refugial groups, resulting in high levels of intrapopulation mtDNA diversity within their former boundaries.

Résumé : Nous avons analysé le polymorphisme de taille de fragments de restriction (RFLP) de l'ADN mitochondrial (ADNmt) pour caractériser l'histoire post-glaciaire complexe du touladi (*Salvelinus namaycush*) et vérifier les hypothèses actuellement avancées au sujet de la dispersion de cette espèce. Nous avons effectué une étude pilote au moyen de 30 enzymes de restriction sur le touladi de 16 populations géographiquement représentatives afin de choisir les caractères apportant de l'information d'intérêt phylogénétique. Après cette étape, nous avons tracé le profil de 1 416 touladis, provenant de 93 populations réparties dans l'ensemble de l'aire de distribution de l'espèce, au moyen de neuf enzymes de restriction variables; cette analyse a révélé que les touladis d'au moins cinq refuges glaciaires ont contribué à la formation des populations actuelles. Nous avons mis en évidence trois grandes lignées d'ADNmt; elles diffèrent suffisamment les unes des autres pour qu'on puisse envisager qu'elles ont divergé au pléistocène moyen. À en juger d'après les différences géographiques et génétiques observées entre deux des lignées étudiées, les avancées glaciaires wisconsiniennes auraient causé une nouvelle divergence vicariante. Contrairement à ce qui s'observe chez les espèces d'eau douce vivant plus au sud, aucune corrélation n'a été mise en évidence entre la proximité géographique des refuges glaciaires et la parenté des lignées d'ADNmt. La distribution actuelle des lignées issues de refuges s'explique facilement par le facteur chronologique ainsi que par le fait que les lacs proglaciaires étaient reliés. Ces lacs ont favorisé une dispersion dans un rayon étendu depuis des refuges multiples, et, plus particulièrement, la dispersion à grande distance des éléments des refuges mississippien et du nord-ouest. Ils ont aussi permis d'importants contacts secondaires entre les groupes des refuges, ce qui explique la grande diversité que présente l'ADNmt à l'intérieur d'une même population dans le territoire qu'occupaient autrefois les lacs proglaciaires. [Traduit par la Rédaction]

Introduction

The Pleistocene glaciations had unprecedented impacts on the ecology and genetic structure of North American species. The genetic and ecological upheavals caused by repeated glacial advances and retreats were especially pronounced among freshwater species, due to their restrictive dispersal requirements (Pielou 1991). Shifting ice fronts frequently altered habitat conditions drastically through the formation and failure of ice dams, drainage shifts, cascading overflows, and sudden emptying or flooding of ice-margin lakes (Dyke and Prest 1987; Pielou 1991). As a result of these large-scale disruptions, species diversities in formerly glaciated areas are far below those observed in neighbouring nonglaciated regions (McAllister et al. 1986). Conversely, the vast proglacial lakes formed from glacial meltwater provided tremendous dispersal opportunities for many aquatic species. The use of these drainage connections as avenues of dispersal has resulted in these taxa having much larger ranges than most species in nonglaciated regions (McAllister et al. 1986).

It is ironic that most studies examining the phylogeographic impacts of Pleistocene glaciations have so far been conducted on species that occur well south of glaciated areas (e.g.,

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Bermingham and Avise 1986; Avise 1992). Within regions formerly covered by the ice sheets, broad-scale phylogeographic studies have been conducted on only a few fish species (e.g., Ward et al. 1989; Bernatchez and Dodson 1991; Billington et al. 1992). Nevertheless, the limited information available suggests that species from glaciated and nonglaciated regions exhibit both quantitative and qualitative differences in genetic structure, with species from glaciated regions having reduced levels of intraspecific divergence and diversity (Bernatchez et al. 1989; Billington and Hebert 1991).

The lake trout (*Salvelinus namaycush*) is an ideal species for studying glacial impacts on phylogeographic structure, as its history and biology are intimately linked to the Pleistocene glaciations. The species' range corresponds closely to glacial limits, and lake trout are largely restricted to deepwater habitats created by glacial scouring (Martin and Olver 1980). The genetic structure of lake trout would have been strongly influenced by Pleistocene events, as repeated glacial cycles would have caused severe reductions in habitat availability. Surviving populations were displaced and isolated into separate refugia for long periods (>50 000 years), providing opportunities for their genetic divergence. Conversely, proglacial lakes present during glacial retreat would have facilitated dispersal and contact among refugial groups. This complex zoogeographic history has made resolution of the species' history difficult and has resulted in numerous, frequently conflicting hypotheses regarding the number and locations of glacial refugia utilized by lake trout (summarized in Crossman and McAllister 1986; Wilson and Hebert 1996). Despite this, lake trout have been used as a model system to explain postglacial dispersal of other aquatic species (Crossman and McAllister 1986; Pielou 1991). Until recently, the general consensus was that lake trout from a Mississippian refuge colonized much of Canada whereas lake trout from Beringian and Atlantic refugia made only local contributions (Black 1983*a*, 1983*b*; Crossman and McAllister 1986). This conclusion was indirectly supported by genetic analyses of lake whitefish (*Coregonus clupeaformis*) that showed phylogeographic patterns concordant with this proposal (Bernatchez and Dodson 1991; Bodaly et al. 1992).

Previous genetic studies of lake trout in eastern Canada have detected several lineages that show considerable geographic structure, which has been attributed to recolonization of this region by fish from several glacial refugia (Grewe and Hebert 1988; Ihssen et al. 1988; Wilson and Hebert 1996). This study extends previous work by using mitochondrial DNA (mtDNA) to examine the impacts of Pleistocene and postglacial events on the genetic structure of lake trout from across the full species range and compares the phylogeographic data obtained with those from other freshwater fishes.

Methods

A total of 1416 lake trout were sampled/collected from 93 populations across the species' range (Table 1), including 30 eastern populations examined previously (Wilson and Hebert 1996). Sample sizes ranged from 3 to 34, with a mean of 15.2 fish per population (Table 1). Where possible, fresh tissues were taken and extracted as described in Wilson and Hebert (1996). For most populations, total DNA (nuclear DNA plus mtDNA) was extracted with an SDS – proteinase K protocol (Bardakci and Skibinski 1994), using 50–100 mg of frozen or ethanol-preserved muscle, liver, or adipose fin tissue per sample.

DNA samples were digested with restriction enzymes and run on 0.8–1.2% agarose gels alongside molecular size standards. The DNA was then denatured, neutralized, and transferred to a nylon membrane (Amersham Hybond-N) by capillary blotting. Membranes were baked at 80°C for 2 h and probed with purified lake trout mtDNA labelled with a chemiluminescent chemical (digoxigenin (DIG), Boehringer Mannheim). Membrane hybridization and visualization were carried out as described by Höltke et al. (1992).

To assess the species' phylogenetic structure, mtDNA from 64 fish from 16 geographically distant populations was screened for variation with 30 restriction enzymes (Table 2). In addition, a 1100 base pair portion of the mtDNA molecule containing the D-loop was amplified using primers and amplification protocol from Bernatchez and Danzmann (1993) and screened for variation with six restriction enzymes that recognize tetranucleotide sequences. Restriction fragment homologies were confirmed using double digests with reference enzymes. Recognition sites were inferred from fragment patterns, as most mutational shifts could be explained by single-site gains or losses. The inferred restriction site data were used to construct a mutational network among haplotypes with the maximum likelihood program RESTML from PHYLIP 3.5c (Felsenstein 1993). The inferred site matrix was also subjected to bootstrapping and majority rule consensus, using the SEQBOOT, MIX, and CONSENSE modules of PHYLIP. Nucleotide divergences among haplotypes were estimated using the formulae of Nei and Tajima (1981) in the REAP computer package (McElroy et al. 1992) and clustered using the neighbour-joining method of Saitou and Nei (1987) offered by the NEIGHBOR program in PHYLIP. Divergence times among mtDNA lineages were estimated using a molecular clock estimate for salmonid mtDNA of 1% per million years (Smith 1992), after correcting for diversity within lineages (Wilson et al. 1985).

The remaining fish were screened with nine restriction enzymes that detected variation (*Ase*I, *Ava*I, *Bam*HI, *Eco*O109I, *Eco*RV, *Hin*dIII, *Sca*I, *Stu*I, and *Sty*I) to determine their phylogeographic origins. Haplotype (nucleon) and nucleotide (π) diversities of populations were calculated using REAP. For nucleotide diversity values calculated from this reduced set of enzymes, it was assumed that enzymes that did not detect more than one haplotype in the initial population screening (Table 3) were invariant in all other populations. Although this assumption may be incorrect, it prevented overinflation of estimated values. Geographic relationships and statistical significance of phylogeographic structure were assessed using the AMOVA program of Excoffier et al. (1992), which partitions variance into within- and among-population components, based on both genetic diversity of populations and relatedness of haplotypes within populations.

Results

Intraspecific phylogeny

Of the 30 restriction enzymes used to screen fish from the 16 test populations (Tables 2 and 3), four enzymes (*Bgl*II, *Cla*I, *Kpn*I, and *Mlu*I) failed to cut lake trout mtDNA. The remaining 26 enzymes recognized an average of 121 cut sites (702 bases) per fish, or 4.2% of the mtDNA genome. Thirteen of these enzymes failed to detect variation among the fish screened and recognized 48 cut sites. Fragment data for these enzymes are presented elsewhere (Wilson 1995).

Thirteen enzymes detected variation (Table 2), revealing 14 haplotypes among fish from the test populations (Table 3). Seven additional haplotypes were detected during the larger geographic survey and were characterized for fragment patterns with all variable enzymes (Table 3). Of the six four-base restriction enzymes used to digest the polymerase chain

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Table 1. Collection sites for lake trout across its native range showing population numbers, geographic location, sample sizes (N), number of haplotypes present (N_h), and nucleon (h) and nucleotide (π) diversities.

No.	Lake	Location	Lat.	Long.	N	N_h	h	π
1	Trouser Lake	Labrador	56°32′	61°46′	14	1	0	0
2	**Chamcook Lake**	New Brunswick	45°09′	67°06′	15	1	0	0
3	Togue Pond	Maine	46°56′	68°53′	11	1	0	0
4	**Moosehead Lake**	Maine	45°40′	69°38′	15	2	0.133	0.009
5	**Long Pond**	Maine	45°58′	70°09′	5	1	0	0
6	Lake Winnepesaukee	New Hampshire	43°36′	71°22′	12	1	0	0
7	Raquette Lake	New York	43°48′	74°36′	15	1	0	0
8	Seneca Lake	New York	42°45′	76°55′	7	2	0.571	0.192
9	Lac Letemplier	Quebec	49°27′	68°47′	20	1	0	0
10	Lac Albanel	Quebec	51°05′	73°00′	12	4	0.455	0.203
11	Lac Mistassini	Quebec	50°30′	73°30′	23	4	0.625	0.246
12	**Lac Beland**	Quebec	48°51′	73°19′	12	1	0	0
13	**Lac Normand**	Quebec	46°29′	73°14′	24	1	0	0
14	Lac Archambault	Quebec	46°19′	74°15′	11	1	0	0
15	Lac des 31 Milles	Quebec	46°12′	75°49′	23	2	0.474	0.156
16	Lac Polonais	Quebec	47°00′	75°22′	7	1	0	0
17	**Lake Opeongo**	Ontario	45°42′	78°23′	30	2	0.129	0.043
18	Clear Lake	Ontario	45°15′	78°32′	30	1	0	0
19	**Tim Lake**	Ontario	45°45′	79°02′	15	1	0	0
20	Elliot Lake	Ontario	46°23′	82°42′	11	2	0.509	0.171
21	**Lake Superior**	Ontario	47°30′	87°00′	21	7	0.784	0.416
22	**Lake Nipigon**	Ontario	49°50′	88°30′	30	9	0.825	0.387
23	Little Sparkling Lake	Ontario	49°50′	90°13′	12	1	0	0
24	Confederation Lake	Ontario	51°05′	92°44′	10	2	0.200	0.013
25	**Daniels Lake**	Ontario	49°55′	93°50′	14	2	0.143	0.102
26	**Scattergood Lake**	Ontario	49°18′	92°43′	11	3	0.691	0.203
27	Lake Mameigweiss	Ontario	49°34′	91°49′	17	2	0.221	0.057
28	Hawley Lake	Ontario	54°30′	84°39′	9	1	0	0
29	Trout Lake	Wisconsin	46°02′	89°49′	17	1	0	0
30	God's Lake	Manitoba	54°40′	94°15′	15	5	0.695	0.323
31	Dymond Lake	Manitoba	58°48′	94°32′	8	4	0.821	0.386
32	Croll Lake	Manitoba	59°34′	98°22′	15	3	0.562	0.324
33	Clearwater Lake	Manitoba	54°05′	101°05′	29	4	0.603	0.267
34	Lake Athapapuskow	Manitoba	54°33′	101°40′	30	6	0.703	0.338
35	Reindeer Lake	Saskatchewan	57°15′	102°15′	20	5	0.784	0.379
36	Little Bear Lake	Saskatchewan	54°20′	104°35′	3	2	0.667	0.341
37	Lac la Ronge	Saskatchewan	55°04′	105°19′	15	5	0.762	0.343
38	Nemieben Lake	Saskatchewan	55°20′	105°20′	19	5	0.790	0.295
39	**Kingsmere Lake**	Saskatchewan	54°06′	106°27′	17	2	0.118	0.031
40	Lac la Plonge	Saskatchewan	55°14′	107°34′	31	4	0.759	0.335
41	Pierce Lake	Saskatchewan	54°30′	109°42′	10	3	0.689	0.382
42	Milliken Lake	Saskatchewan	59°27′	108°45′	3	1	0	0
43	Lake Athabasca	Saskatchewan	59°15′	109°15′	10	3	0.711	0.401
44	Cornwall Lake	Alberta	59°36′	110°35′	9	2	0.500	0.130
45	Peerless Lake	Alberta	56°37′	114°40′	19	2	0.351	0.227
46	Swan Lake	Alberta	52°07′	115°10′	20	1	0	0
47	Rock Lake	Alberta	53°27′	118°15′	20	1	0	0
48	Waterton Lake	Alberta	49°03′	113°54′	13	2	0.513	0.329
49	Cosley Lake	Montana	49°29′	114°00′	18	1	0	0
50	**Twin Lake**	Montana	46°00′	113°00′	5	1	0	0
51	Moose Lake	British Columbia	53°00′	119°00′	32	1	0	0
52	Francois Lake	British Columbia	54°03′	125°45′	34	1	0	0
53	Burnt Rose Lake	British Columbia	59°00′	128°00′	8	1	0	0
54	Graveyard Lake	British Columbia	59°00′	127°00′	5	1	0	0
55	**Muncho Lake**	British Columbia	58°59′	125°47′	20	1	0	0
56	Summit Lake	British Columbia	58°40′	124°20′	12	1	0	0
57	Nueltin Lake	Northwest Territories	60°30′	99°30′	11	3	0.655	0.352

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Table 1 (*concluded*).

No.	Lake	Location	Lat.	Long.	N	N_h	h	π
58	Hawk Lake	Northwest Territories	63°38′	90°40′	8	1	0	0
59	Lailor Lake	Northwest Territories	69°17′	82°50′	27	1	0	0
60	Grinnell Lake	Northwest Territories	69°34′	83°55′	15	1	0	0
61	Sarcpa Lake	Northwest Territories	68°32′	83°15′	29	1	0	0
62	Hall Lake	Northwest Territories	68°40′	82°30′	23	1	0	0
63	Pelly Bay	Northwest Territories	68°33′	89°47′	11	1	0	0
64	Spence Bay	Northwest Territories	69°34′	93°30′	8	1	0	0
65	Unnamed lake	Northwest Territories	69°09′	104°43′	5	1	0	0
66	Unnamed lake	Northwest Territories	69°07′	105°07′	16	1	0	0
67	Great Slave Lake	Northwest Territories	61°23′	115°38′	27	4	0.658	0.277
68	Great Bear Lake	Northwest Territories	66°00′	119°00′	5	2	0.400	0.205
69	Fish Lake	Northwest Territories	71°50′	124°33′	14	1	0	0
70	Raddi Lake	Northwest Territories	71°41′	123°43′	14	1	0	0
71	Itkrilek Lake	Northwest Territories	69°33′	132°05′	12	1	0	0
72	Tassiruak Lake	Northwest Territories	69°37′	132°12′	12	1	0	0
73	Sitidgi Lake	Northwest Territories	68°32′	132°40′	25	1	0	0
74	Unnamed lake	Northwest Territories	69°17′	133°52′	6	1	0	0
75	Peter Lake	Northwest Territories	68°46′	134°08′	11	1	0	0
76	Noell Lake	Northwest Territories	68°32′	133°34′	21	1	0	0
77	Frances Lake	Yukon Territory	61°23′	129°35′	12	1	0	0
78	Ethel Lake	Yukon Territory	63°22′	136°05′	14	1	0	0
79	Teslin Lake	Yukon Territory	60°15′	132°58′	17	1	0	0
80	Quiet Lake	Yukon Territory	61°05′	133°05′	10	2	0.467	0.239
81	Marsh Lake	Yukon Territory	60°25′	134°18′	15	2	0.476	0.244
82	Tagish Lake	Yukon Territory	60°10′	134°20′	13	3	0.410	0.154
83	Lake Laberge	Yukon Territory	61°11′	135°12′	14	1	0	0
84	Fish Lake	Yukon Territory	60°37′	135°14′	11	1	0	0
85	Braeburn Lake	Yukon Territory	61°27′	135°48′	11	1	0	0
86	Kusawa Lake	Yukon Territory	60°20′	136°22′	15	2	0.133	0.009
87	Dezadeash Lake	Yukon Territory	60°28′	136°59′	11	2	0.509	0.260
88	Aishihik Lake	Yukon Territory	61°26′	137°15′	13	1	0	0
89	**Kluane Lake**	Yukon Territory	61°15′	138°45′	13	1	0	0
90	**Toolik Lake**	Alaska	68°38′	149°36′	20	1	0	0
91	Island Lake	Alaska	68°31′	149°32′	14	1	0	0
92	Galbraith Lake	Alaska	68°28′	149°25′	8	1	0	0
93	Ugashik Lake	Alaska	56°48′	156°52′	12	1	0	0

Note: The 16 lakes shown in bold type were used in the initial screening for geographically informative restriction enzymes.

reaction (PCR) amplified D-loop region, only *Dde*I and *Sty*I detected more than one haplotype (Table 2). The *Sty*I polymorphism in the PCR-amplified fragment coincided with the polymorphism detected by digestion of the entire mtDNA molecule, localizing this variable site. *Dde*I fragment patterns were not included in Table 4, as only fish with "B" patterns for *Ava*I and *Bam*HI were routinely screened with this enzyme; random sampling and digestion of mtDNA from fish from other lineages revealed only the *Dde*I "A" pattern.

The mutational network constructed from the inferred restriction sites (Fig. 1) revealed three major groups distinguishable by two or more restriction site differences that corresponded to lineages observed in earlier studies (Grewe and Hebert 1988; Wilson and Hebert 1996). Haplotypes 1–4 and 15–19 corresponded to group A of Grewe and Hebert (1988) and differed from all other haplotypes by at least three mutational steps, excepting haplotype 4, which differed from haplotype 13 by only two steps. Haplotypes 5–7 corresponded to group B of Grewe and Hebert (1988) and were distinguishable from all other haplotypes by unique *Dde*I and *Sty*I fragment patterns (Fig. 1). Haplotypes 8–12, 14, and 20 differed from all other haplotypes by a minimum of seven mutational steps (Fig. 1) and belong to Grewe and Hebert's (1988) C lineage. Haplotypes 13 and 21 were very similar to group B haplotypes except for their *Dde*I and *Sty*I fragment patterns. These two haplotypes were assigned their own group (D), as they differed from groups A, B, and C by at least two restriction site characters. Bootstrap analysis supported two clades with 100% certainty, group C versus A, B, and D (Fig. 2). Reduced bootstrap separation of group A from groups B and D (78%) was caused by the near-intermediacy of haplotype 4, which occurred as one individual in Lake Superior, Ontario. Groups A, B, and D were nonetheless considered valid, as each group could be distinguished by two or more restriction site characters. Divisions within group C lacked significant structure, due to unresolved *Ase*I and *Ava*I homoplasies.

Estimated pairwise nucleotide divergences (±1 SE) among haplotypes ranged from 0.07 to 1.03%, with a mean value of 0.54%. Groups B and D differed by 0.24% (±0.02) (Fig. 2) and showed limited divergence from group A haplotypes (0.42%

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Table 2. Haplotypes for restriction enzymes that detected variation in lake trout mtDNA and their resulting fragment patterns showing fragment sizes in kilobases.

***Ase*I**	
A	3.02 2.93 2.75 2.19 2.00 1.75 1.75 0.57
B	3.02 2.75 2.19 2.15 2.00 1.75 1.75 0.80 0.57
C	2.93 2.75 2.19 2.10 2.00 1.75 1.75 1.49
D	4.40 3.02 2.93 2.19 2.00 1.75 0.57
***Ava*I**	
A	5.49 2.68 2.22 2.12 1.68 1.36 1.10 0.57
B	5.49 2.68 2.22 1.68 1.52 1.36 1.10 0.60 0.57
C	5.49 2.68 1.84 1.68 1.52 1.36 1.10 0.60 0.57 0.37
D	5.49 2.68 1.84 1.68 1.36 1.10 0.90 0.62 0.60 0.57 0.37
***Bam*HI**	
A	16.80
B	9.48 7.32
C	9.48 5.00 2.32
*Bsp*HI	
A	10.10 3.41 3.24
B	10.10 6.65
*Bst*EII	
A	9.46 7.17
B	9.46 6.55 0.68
***Eco*O109I**	
A	4.39 3.98 2.75 1.17 0.73 0.67 0.64 0.40 0.30
B	4.39 3.15 2.75 1.17 0.84 0.73 0.67 0.64 0.40 0.30
C	4.71 4.39 2.75 1.17 0.67 0.64 0.40 0.30
D	4.39 4.20 2.75 1.17 0.67 0.64 0.40
***Eco*RV**	
A	14.25 1.55 0.96
B	15.80 0.96
C	14.25 2.52
***Hind*III**	
A	9.10 2.24 1.96 1.76 1.48 0.26
B	9.10 3.44 2.24 1.76 0.26
C	9.10 2.24 1.96 1.76 1.74
*Nco*I	
A	7.46 6.83 1.83 1.19
B	8.29 7.46 1.83
***Sca*I**	
A	10.20 6.57
B	6.57 5.35 4.84
C	11.95 4.84
D	16.80
*Sph*I	
A	12.97 3.86
B	9.85 3.86 3.12
***Stu*I**	
A	5.10 2.36 1.68 1.55 1.22 1.04 0.54 0.49 0.45 0.31
B	5.10 2.36 1.68 1.55 1.35 1.22 0.54 0.49 0.45
C	5.10 2.36 1.68 1.55 1.35 0.82 0.54 0.49 0.45 0.40
***Sty*I**	
A	2.20 1.30 1.05 1.00 0.85 0.80 0.74 0.70 0.56 0.52 0.48 0.36 0.25
B	2.20 1.05 1.00 0.85 0.83 0.80 0.74 0.70 0.56 0.52 0.48 0.46 0.36 0.25
D-loop	
***Dde*I**	
A	0.62 0.57
B	0.62 0.27 0.20
***Sty*I**	
A	0.78 0.28
B	1.10

Note: Enzymes used for geographic survey of lake trout populations are indicated in bold type.

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Table 3. Composite haplotypes for restriction enzyme polymorphisms detected among lake trout populations showing locations of individuals with each haplotype among 16 test populations, and additional haplotypes detected during the geographic survey.

Haplotype	Composite fragment pattern														Location
Test populations															
1	A	A	A	A	A	A	A	A	A	A	A	A	A	A	17, 21, 22, 25, 26, 39
2	A	A	A	A	A	A	A	B	A	A	A	A	A	A	19
3	A	A	A	A	B	A	A	A	A	A	A	A	A	A	22
4	A	A	B	A	A	A	A	A	A	A	A	A	A	A	21
5	A	B	B	A	A	A	A	A	A	A	B	A	B	B	2, 4, 5, 12, 13, 17, 21, 22
6	B	B	B	A	A	A	A	A	A	A	B	A	B	B	22
7	A	B	C	A	A	A	A	A	A	A	B	A	B	B	4
8	B	C	C	B	A	B	B	A	A	B	B	B	A	A	21, 22, 25, 26
9	A	C	C	B	A	B	B	A	A	B	B	B	A	A	21, 22, 90
10	B	C	C	B	A	B	B	A	B	B	B	B	A	A	21
11	B	D	C	B	A	B	B	A	A	B	B	B	A	A	22
12	A	D	C	B	A	B	B	A	A	B	B	B	A	A	21, 22
13	A	B	B	A	A	A	A	A	A	A	B	B	A	A	21, 22, 26, 39, 55, 89
14	A	C	C	B	A	B	B	A	A	C	B	A	A	A	21, 22, 50
Additional haplotypes															
15	A	A	A	A	A	A	A	A	A	D	A	A	A	A	33
16	A	A	A	A	A	C	A	A	A	A	A	A	A	A	24
17	C	A	A	A	A	A	A	A	A	A	A	A	A	A	11
18	D	A	A	A	A	A	A	A	A	A	A	A	A	A	30
19	A	A	A	A	A	A	C	A	A	A	A	A	A	A	37, 38
20	A	C	C	B	A	B	B	A	A	B	B	C	A	A	34
21	A	B	B	A	A	D	A	A	A	A	B	B	A	A	82, 86

Note: Enzymes from Table 2 are listed in order (*Ase*I, *Ava*I, *Bam*HI, *Bsp*HI, *Bst*EII, *Eco*O109I, *Eco*RV, *Hin*dIII, *Nco*I, *Sca*I, *Sph*I, *Stu*I, and *Sty*I), as well as *Dde*I digestion of the amplified D-loop. Names for locations are given in Table 1.

Table 4. Haplotypes detected during the geographic survey with nine informative restriction enzymes (*Ase*I, *Ava*I, *Bam*HI, *Eco*O109I, *Eco*RV, *Hin*dIII, *Sca*I, *Stu*I, and *Sty*I) showing abundances and locations for uncommon haplotypes.

Haplotype	Composite fragment pattern									*N*	Location
A1	A	A	A	A	A	A	A	A	A	270	(31)
A2	A	A	A	A	A	B	A	A	A	15	19
A3	A	A	A	C	A	A	A	A	A	5	24, 40
A4	C	A	A	A	A	A	A	A	A	2	11
A5	D	A	A	A	A	A	A	A	A	1	30
A6	A	A	A	A	C	A	A	A	A	9	35, 37, 38
A7	A	A	B	A	A	A	A	A	A	1	21
A8	A	A	A	A	A	A	D	A	A	1	33
B1	A	B	B	A	A	A	A	A	B	205	(20)
B2	B	B	B	A	A	A	A	A	B	1	22
B3	A	B	C	A	A	A	A	A	B	1	4
C2	A	C	C	B	B	A	B	B	A	411	(40)
C1	B	C	C	B	B	A	B	B	A	51	(13)
C3	A	C	C	B	B	A	C	A	A	39	(8)
C4	A	C	C	B	B	A	B	C	A	1	34
C5	B	D	C	B	B	A	B	B	A	1	22
C6	A	D	C	B	B	A	B	B	A	7	21, 22, 34
D1	A	B	B	A	A	A	A	B	A	391	(44)
D2	A	B	B	D	A	A	A	B	A	28	2, 86

Note: Numbers of populations containing common haplotypes are given in parentheses. Locations of other haplotypes indicate populations listed in Table 1.

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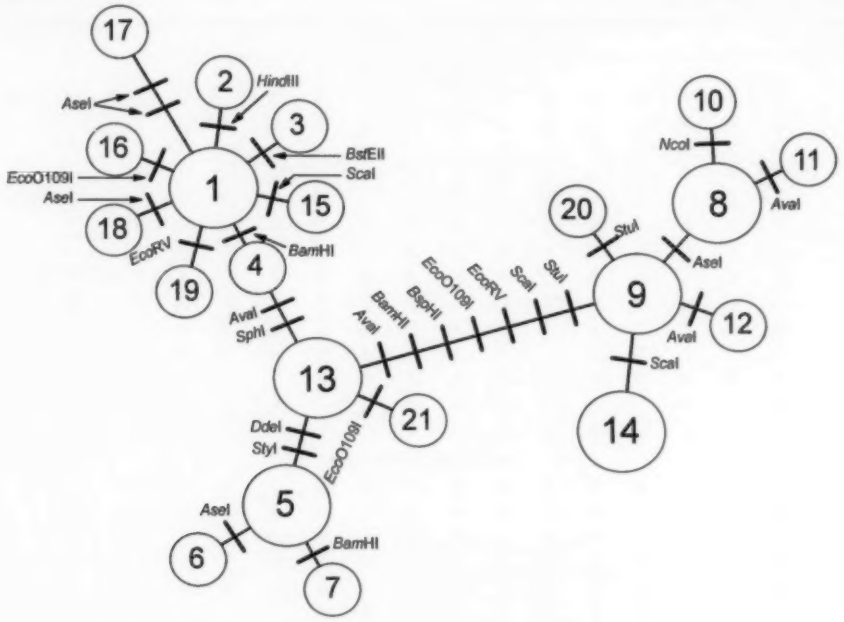
Fig. 1. Parsimony network showing mutational relationships among lake trout haplotypes listed in Table 3. Larger ellipses indicate abundant haplotypes (>30 individuals).



(±0.02)). Group C was the most divergent, differing from group A by 0.88% (±0.01) and from groups B and D by 0.83% (±0.02). Mean divergence within each lineage was equal to or less than 0.15% (Fig. 2).

Geographic structure

The geographic survey of the remaining populations with nine informative enzymes revealed that groups A, B, C, and D had differential geographic distributions with considerable overlap (Fig. 3). There was little correspondence between geographic distance and genetic divergence, with closely related lineages showing largely allopatric distributions. Group B fish occurred from central Ontario to the Atlantic coast and dominated populations in Quebec, New Brunswick, and New England. By contrast, lake trout with group D haplotypes dominated southern Alaska and Yukon populations and occurred in populations throughout central Canada, extending as far east as western Ontario. Groups B and D were both present in Lake Nipigon and Lake Superior, which formed the western limit for lake trout belonging to group B (Fig. 3). The origin of group D fish in Waterton Lake, Alberta, is uncertain, as this lake has been stocked with fish from Swan Lake (D. Donald, Environment Canada, Regina, Sask., personal communication). Group A fish occurred throughout central Canada, from Quebec to Alberta, and into the southern Northwest Territories (Fig. 3). Group C was widespread throughout northern Alaska and arctic Canada, as well as inland throughout central Canada (Fig. 3), with disjunct occurrences in Montana, southern British Columbia, Alberta, and Lakes Albanel and Mistassini in western Quebec.

The majority of rare haplotypes differed from common haplotypes by single mutations (Fig. 1; Tables 3 and 4) and typically occurred as single individuals in large lakes such as Nipigon and Superior, although several haplotypes (A2, A3, A6, and C6) were either common within single sites or occurred in several populations (Table 4). The three most common group C haplotypes (C1, C2, and C3) showed strong geographic patterning, although all three haplotypes cooccurred in central Canada (Fig. 4). Lake trout with haplotype C1 occurred primarily in Ontario, Quebec, and Manitoba, with Great Slave Lake as the northwest limit to their observed distribution, whereas haplotype C2 dominated populations in arctic and western Canada (Fig. 4). Haplotype C3 fish were largely limited to lakes in Montana and southwest Alberta (Waterton Lake), but also occurred sporadically in Saskatchewan, Manitoba, and western Ontario (Fig. 4).

Contact among lineages was extensive, particularly among groups A, C, and D (Fig. 3). All three of these groups cooccurred throughout central Canada, extending northwest into Lake Athabasca and Great Slave Lake. Groups C and D showed the most extensive codistributions, with additional sympatry in Great Bear Lake, the Mackenzie Delta, and the southern Yukon Territory. Groups B and D showed virtually no overlap, co-occurring only as individuals in Lakes Albanel, Nipigon, and Superior (Fig. 3).

Variation of both nucleon and nucleotide diversity within populations was largely influenced by the number of major lineages present. High levels of diversity occurred almost exclusively within areas formerly covered by major proglacial lakes (Fig. 5). The majority of populations within former lake margins contained haplotypes from more than one mtDNA lineage, with the greatest regional diversity occurring in populations within or near areas formerly covered by Lakes Algonquin, Agassiz, and McConnell (Fig. 5). By contrast, virtually all populations beyond the former proglacial lake margins contained only single haplotypes. This relationship held true even

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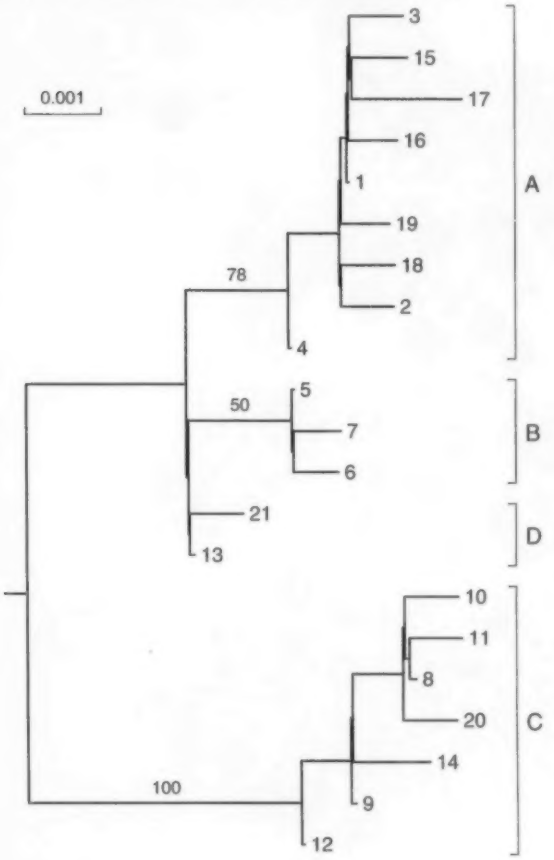
Fig. 2. Neighbour-joining dendrogram of estimated nucleotide divergences among lake trout haplotypes within geographically representative populations showing divergences among phylogenetic groups described in the text. Numbers above branches represent percent bootstrap support based on 10 000 replications. Scale bar indicates nucleotide divergence of 0.001 (0.1%).



for apparently anomalous populations in the Yukon Territory (Fig. 5), where populations with diversities greater than zero occurred within the former boundaries of glacial Lake Champagne (Lindsey et al. 1981).

The significance of genetic heterogeneity among populations and regions was assessed with the AMOVA program (Excoffier et al. 1992), using two predictive models of regional partitioning to test phylogeographic structure. Populations were partitioned on the basis of modern drainage basins (Arctic, Hudson Bay, Atlantic, Gulf of Mexico, and Pacific drainages) and by postulated refugia (Atlantic, Mississippian, Missourian, and two Beringian refugia) based on earlier multiple-refugia hypotheses (Lindsey 1964; Khan and Qadri 1971; Black 1983*a*, 1983*b*). Both models indicated significant geographic structuring ($p < 0.001$) within and among populations and regions. The refuge model was more effective than the drainage model in partitioning variance components such that variance among regions was maximized ($V_A = 1.42$ (54%) versus 0.71 (29%)) and variance within regions was minimized ($V_B = 0.49$ (19%) versus 1.03 (42%)), indicating that the refuge model best explained geographic patterns of genetic variation. This suggests that the observed genetic structure reflects historical rather than present-day events and that current conditions do not favour gene flow among populations. Much of the difference between the two models resulted from the occurrence of several genetic lineages within the Atlantic and Hudson Bay drainages whereas refugial delineations suggested by Khan and Qadri (1971) and Black (1983*a*, 1983*b*) were largely dominated by single clades (Fig. 3). Both models had high levels of variation in central Canada (Hudson Bay drainage – Mississippian refuge), reflecting the extensive admixture of multiple genetic groups. This highlights the extensive dispersal of lake trout from refugia via proglacial lakes (Fig. 6) and provides an explanation for the many conflicting interpretations of lake trout postglacial dispersal among previous studies.

Discussion

Intraspecific divergence

Levels of diversity within and among lake trout mtDNA lineages (<1%) are consistent with data from other northern freshwater species (Bernatchez and Dodson 1991; Billington and Hebert 1991; Wilson et al. 1996) and are probably due to repeated glacial disturbances causing reduced mtDNA diversity through habitat loss, displacement, and persistence in suboptimal habitats during glacial events (Avise et al. 1984). The limited divergence among lineages and low diversity within each group suggest their origin during the Pleistocene. Based on a mtDNA divergence rate of 1% per million years (Smith 1992), group C diverged from other lake trout lineages about 450 000 – 700 000 years ago. This may be an underestimate, however, as the calculation assumes a highly diverse ancestral population (Wilson et al. 1985). In addition, slower estimates for salmonid mtDNA divergence such as 0.5–0.9% per million years (Martin and Palumbi 1993) suggest longer divergence times. Using the lower bound of Martin and Palumbi's estimate, divergence among lake trout lineages could potentially have occurred as early as the beginning of the Pleistocene epoch, about 1.6 million years ago. Although divergence times were not estimated for groups A, B, and D, their levels of genetic divergence suggest that these lineages all diverged well before the Wisconsinan glaciation.

The extent of genetic divergence among the lineages showed no clear correlation with geographic distance. Mitochondrial lineages B and D were closely related, but occupied opposite ends of the continent. Similarly, the three most common group C haplotypes differed by only single cut sites, but had largely disjunct distributions. These groups probably originated from mid-Pleistocene populations that were subdivided by glacial advances, with subsequent divergence in separate refugia through either mutation or lineage extinction. Given the close relationship of the species' range to Pleistocene glacial limits, this cycle of displacement, divergence, and recolonization has probably occurred repeatedly and been a key factor in the formation and differentiation of the major lake trout lineages.

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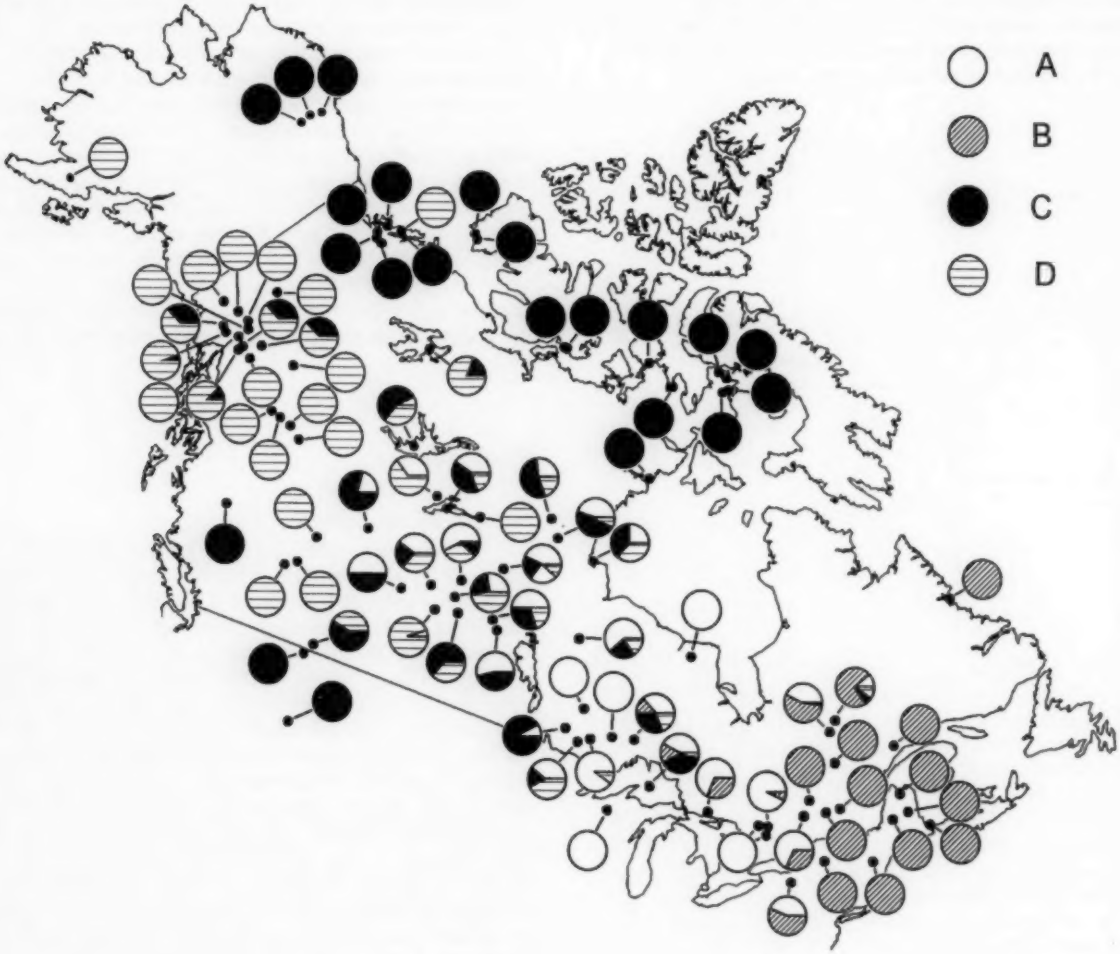
Fig. 3. Distributions of mtDNA lineages A, B, C, and D among sampled lake trout populations. Populations and sample sizes are listed in Table 1.



Refugial origins and postglacial dispersal

The geographic distributions of the major lake trout mtDNA lineages strongly suggest their persistence in and dispersal from separate refugia, supporting previous multiple-refugia hypotheses (Lindsey 1964; Khan and Qadri 1971). Groups A, B, and D appear to have each dispersed from single refugia (Fig. 6). Group A fish appear to have dispersed from a Mississippian refuge and group B from an Atlantic source (Wilson and Hebert 1996). These major refugia have been documented as sources for an array of species with widely varying distributions and ecology (Crossman and McAllister 1986), including lake trout (Lindsey 1964; Khan and Qadri 1971; Black 1983*a*, 1983*b*).

Group D lake trout apparently dispersed from a southern Beringian refuge (Fig. 3). Although much of Alaska was ice-free and could have served as a refuge, the absence of lake trout from the lower Yukon River in Alaska argues against this (Lindsey 1964). The Nahanni Valley in the western Northwest Territories is a likely refugial location, as it formed the terminus of a valley between the Cordilleran and Laurentide ice sheets and contained periglacial lakes throughout the Wisconsinan glaciation (Dyke and Prest 1987). The probable use of a Nahanni refuge is supported by group D's virtual absence from the Yukon and Alaska north slope, as well as its abundance in southern Yukon populations (Fig. 3). A Nahanni refuge has previously been proposed for lake whitefish based on allozyme data (Foote et al. 1992), and the same refuge may also have been important for other taxa (Lindsey and McPhail 1986).

Geographic patterning within group C was more complex and suggests vicariant disruption by glacial advances, with the three most common haplotypes dispersing from separate refugia (Fig. 4). The distribution of haplotype C1 (Fig. 4) can best be explained by its dispersal from a southern (Mississippian or Missourian) refuge, as its distribution is strongly concordant with group A fish from the Mississippian refuge (Fig. 3). It is therefore probable that group C fish in Quebec (Figs. 3 and 4) came from a southern rather than a Beringian

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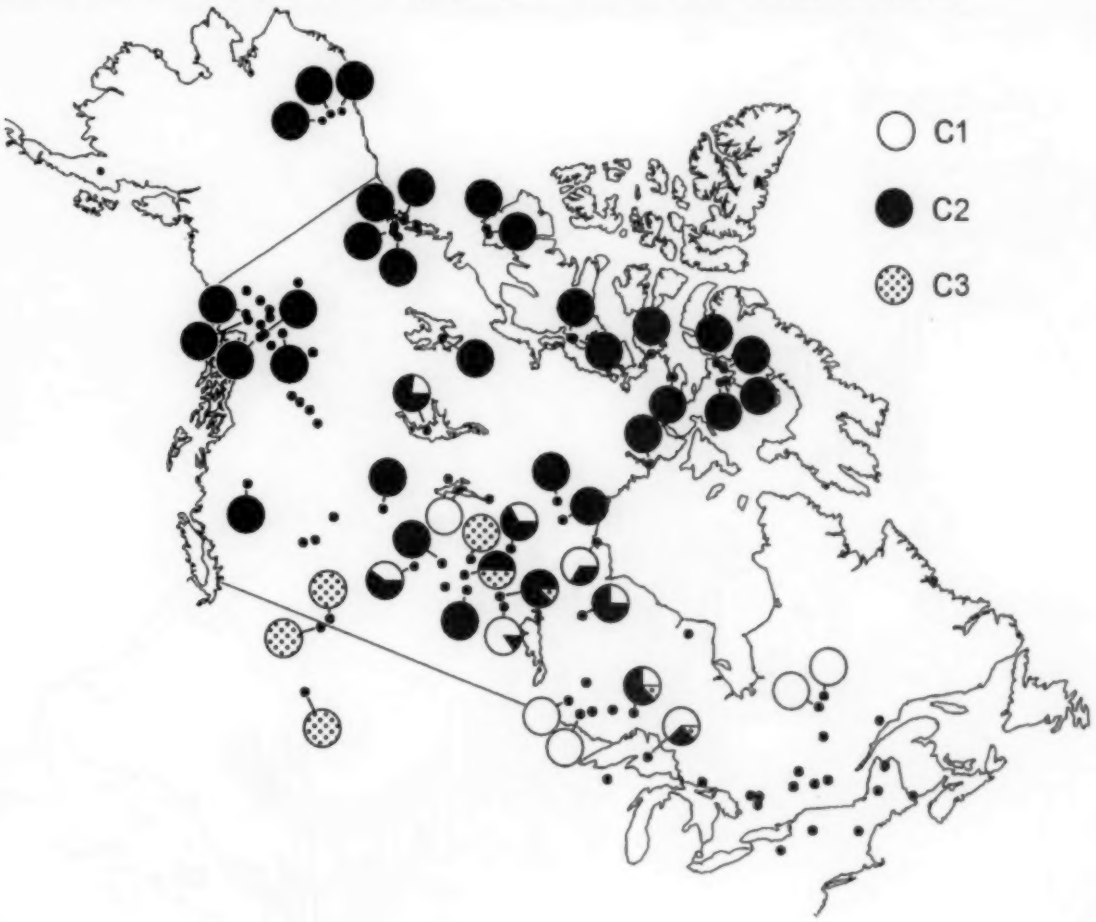
Fig. 4. Distributions of the common group C haplotypes (C1, C2, and C3). Dots indicate sites where group C haplotypes are absent. Pies indicate relative and not absolute proportions of these haplotypes; presence–abundances of other haplotypes are not shown.



source, as previously suggested by Wilson and Hebert (1996). By contrast, haplotype C2 and derivative fish appear to have persisted in a Beringian refuge separate from that occupied by group D, as the two lineages show largely separate arctic distributions (Fig. 3).

The localized distribution of haplotype C3 in western Montana and southwestern Alberta suggests its persistence in a nearby refuge, as suggested by Lindsey (1964). This lineage does not appear to have utilized a Missourian refuge, as emigrants from this system would have had early access to Lake Agassiz with subsequent widespread dispersal. Crossman and McAllister (1986) differentiated between a Missourian refuge and potential refugia in western Montana and Alberta, based on the presence of several endemic and cold-water fish and invertebrate taxa. Additional evidence for a cold-water refuge in this region comes from fossil remains of Arctic grayling (*Thymallus arcticus*) from January Cave in southwestern Alberta, dated at 23 000 – 31 000 years ago (Burns 1991).

The geographic distribution of mitochondrial diversity suggests that lake trout dispersal was primarily dependent on the network of proglacial lakes that spanned much of Canada, although headwater exchange and coastal dispersal were also important (Figs. 5 and 6). Dispersal of group B lake trout from their Atlantic refuge throughout eastern Canada and New England occurred primarily via regional access through glacial Lakes Vermont and Ojibway-Barlow and by following retreating ice margins (Wilson and Hebert 1996). Colonization of Ontario by group B occurred via a connection between glacial Lakes Iroquois and Vermont, and thence into the Laurentian Great Lakes. The distribution of Mississippian (group A) lake trout in eastern North America approximates that of the proglacial lakes in the Great Lakes basin such as glacial Lakes Algonquin and Iroquois (Dyke and Prest 1987). Similarly, Mississippian lake trout had repeated intermittent access to Lake Agassiz 12 800 to 9000 years ago (Dyke and Prest 1987) and colonized western Canada via Lakes Agassiz and McConnell, probably reaching Lake McConnell through a direct connection during the Emerson phase of Lake Agassiz 9900–9500 years ago (Smith and Fisher 1993). Although Mississippian lake trout would have been able to disperse eastward from Lake McConnell 9000–8000 years age as ice margins retreated (Black 1983*a*; Dyke and Prest 1987), the absence of

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Fig. 5. Geographic distribution of diversity within lake trout populations with respect to former maximal extents of major proglacial lakes (Ojibway-Barlow, Algonquin, Iroquois, Agassiz, Peace, and McConnell) indicated by shaded areas. White and black bars represent values of nucleon and nucleotide diversities from Table 1, respectively. Dots represent populations with single haplotypes only.

group A haplotypes in regions northwest of Great Slave Lake (Fig. 3) suggests that Mississippian lake trout were not the primary colonists of the Mackenzie Delta, in contrast with earlier expectations (Black 1983*a*; Crossman and McAllister 1986).

Glacial retreat and ensuing dispersal opportunities began much earlier in western Canada than in the east. Dispersal of lake trout from the two northwestern refugia probably began soon after the glacial maximum 18 000 years ago (Dyke and Prest 1987), as ice retreat was rapid in the northwest and much of the Yukon and Alaska was deglaciated by 14 000 years ago. The resulting meltwater would have facilitated lake trout dispersal both inland via proglacial lakes and along the Arctic coast by reducing salinity to levels tolerated by lake trout (Martin and Olver 1980). Beringian lake trout dispersing along the Arctic coast would have followed glacial margins closely, gradually colonizing eastward until the demise of the Laurentide ice sheet about 6000 years ago (Dyke and Prest 1987). Similar dispersal patterns have been observed for other Beringian emigrants such as Arctic char (*Salvelinus alpinus*) (Wilson et al. 1996). Evidence from other populations indicates that Beringian group C lake trout dispersed along the coast at least as far as the Melville Peninsula (Wilson and Hebert 1993). Coastal movements may have also facilitated inland dispersal, as dispersal from a Beringian refuge by many species is thought to have been prevented by a barrier waterfall on the lower Mackenzie River that lasted from 11 500 to 6100 years age (Lindsey and McPhail 1986). Based on the distributions of groups C and D in eastern Canada (Figs. 3 and 4), it is certain that Beringian- and Nahanni-refuge lake trout were able to colonize glacial Lake McConnell by 9500 years ago, with subsequent dispersal into Lake Agassiz (Smith and Fisher 1993). Movement of Beringian group C fish into southern Yukon lakes probably resulted from connections between the Liard River and glacial Lake Champagne about 10 000 years ago, as this proglacial lake covered four of the

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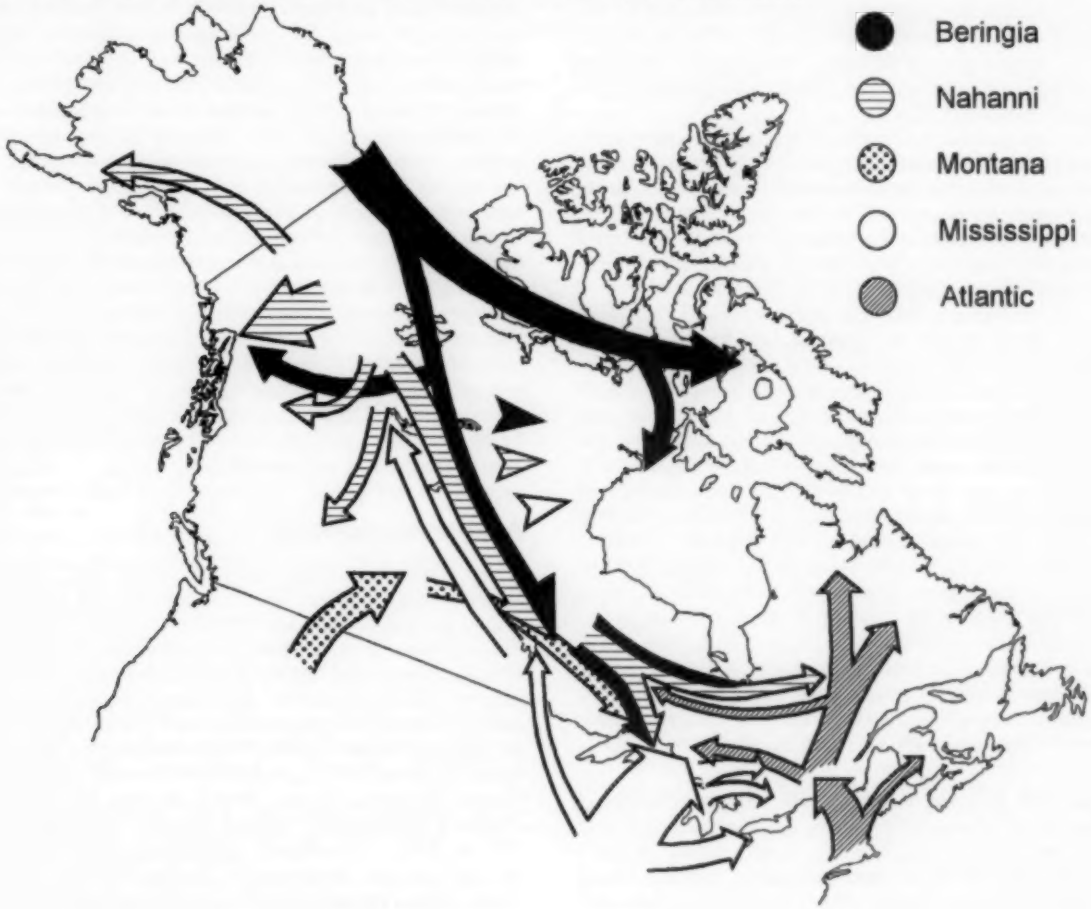
Fig. 6. Hypothesized refugial origins and dispersal for extant lake trout populations based on distributions of mitochondrial haplotypes shown in Figs. 3 and 4 and geological data of proglacial lake locations and connections (Dyke and Prest 1987).



five lake basins where group C fish were detected (Lindsey et al. 1981). Dispersal through the southern Yukon Territory and northern British Columbia was similarly governed by a complex series of ice movements and drainage transfers (Bodaly and Lindsey 1977; Lindsey and McPhail 1986). Dispersal of group D lake trout from the Nahanni refuge into southern Alaska probably occurred via drainage reversals in the Peel River system during early stages of deglaciation (Bodaly and Lindsey 1977). The eastward dispersal of group D lake trout mirrored that of Beringian group C emigrants (Fig. 6). Both groups reached the Great Lakes region (Figs. 3, 4, and 6), and the presence of group D lake trout in Lake Albanel (Fig. 3) indicates that some of these fish were able to colonize Quebec via Lake Ojibway-Barlow.

Dispersal of lake trout from the Montana refuge appears to have been quite limited (Figs. 4 and 6). Suboptimal dispersal conditions were caused by ice stagnation and low volumes of meltwater as well as a shortage of suitable habitats (Christiansen 1979; Dyke and Prest 1987). Proglacial lakes and drainages were localized and laden with glacial till and were subject to catastrophic outburst floods which frequently drained the lakes completely (Christiansen 1979). Lake trout are poorly suited to such conditions (Martin and Olver 1980), although the presence of haplotype C3 in Saskatchewan, Manitoba, and western Ontario indicates that some fish were able to disperse into Lake Agassiz, probably via glacial Lake Saskatchewan, which existed from 14 000 to 11 000 years ago and was connected to Lake Agassiz 11 500 – 11 000 years ago (Christiansen 1979).

Secondary contact among refugial groups

The striking variation in mtDNA diversity among lake trout populations is largely due to the number of refugial lineages present. The close relationship between the geographic distribution of diverse populations and former proglacial lakes (Fig. 5) strongly suggests secondary contact among refugial groups within these lakes as the cause for intrapopulation diversity. By contrast, populations of lake trout beyond proglacial lake limits were probably founded by relatively few individuals from single refugial sources.

This study makes it clear that secondary contact among groups was extensive throughout central Canada. The

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co-occurrence of all of the refugial groups in Lakes Superior and Nipigon (Figs. 3 and 4) underscores the tremendous dispersal facilitated by postglacial drainage events (Fig. 6) and highlights the importance of the major proglacial lakes in facilitating transcontinental dispersal. The dispersal opportunities provided by these meltwater lakes were unparalleled in nonglaciated habitats (Fig. 6).

The correspondence between proglacial lake connections and haplotype distributions suggests that colonization order significantly influenced modern abundances of lake trout refugial groups. As the probabilities for persistence of mtDNA haplotypes within populations are proportional to their initial abundances (Birky et al. 1983), the rapid expansion of population sizes by early colonists in newly formed habitats would place later arrivals at a numerical disadvantage. The predominance of the Beringian and Nahanni lake trout lineages in northwestern Canada would therefore have been assured by their early dispersal into glacial Lakes Peace and McConnell. However, late arrival times could potentially be offset by large number of colonists. For example, despite the likelihood that Mississippian lake trout were present in Lake Agassiz prior to its connection with Lake McConnell, the large numbers of Beringian and Nahanni refuge fish in Lakes Peace and McConnell would have ensured their current abundances in lakes within the former Lake Agassiz basin. By contrast, the low numbers of lake trout with haplotype C3 in central Canada indicate that fish from the Montana refuge had little impact on the genetic composition of proglacial lake populations, due to either low numbers of colonists or their late arrival. At the nuclear level, however, genetic contributions of each group could diffuse through proglacial populations via random mating events (Birky et al. 1983; Billington and Hebert 1991).

Comparative patterns of intraspecific phylogeography

Comparison of intraspecific nuclear and mitochondrial data shows that impacts of drainage events during glacial–interglacial transitions on phylogeographic patterns varied considerably among species. For example, allozyme and mtDNA studies of lake trout from Ontario and Manitoba yielded opposing zoogeographic interpretations. Allozyme data suggested two relatively homogeneous major geographic groups that originated from Mississippian and Atlantic refugia (Ihssen et al. 1988) whereas the mtDNA data show the sympatric occurrence of fish from four out of the five refugial groups in this region. The extensive secondary contact among refugial groups resulted in the homogenization of nuclear markers among previously isolated groups, indicating the geographic extent of these contact zones, rather than distributions of refugial groups themselves as proposed by Ihssen et al. (1988). Combined allozyme–mtDNA analysis of walleye (*Stizostedion vitreum*) populations similarly showed that mtDNA was more effective than allozymes in elucidating phylogeographic patterns, due to reduced signal from nuclear characters as a result of secondary contact among refugial groups (Ward et al. 1989). By contrast, separate allozyme and mtDNA phylogeographic analyses of lake whitefish (Bernatchez and Dodson 1991; Bodaly et al. 1992; Foote et al. 1992) produced nearly identical interpretations of postglacial dispersal, with widespread distribution of Mississippian fish and only localized contributions from other refugia, with little or no intergradation of geographic races.

The extent and timing of proglacial lakes as well as connections and conditions among them produced qualitative differences in dispersal opportunities and resulting phylogeographic structure among the species comprising regional faunas (Ward et al. 1989; Bernatchez and Dodson 1991; Wilson and Hebert 1996). In contrast with phylogeographic concordance among aquatic species from nonglaciated areas (Bermingham and Avise 1986; Avise 1992), phylogeographic patterns among northern species show marked differences that can readily be interpreted in light of postglacial history and species-specific ecological characteristics. It is becoming clear that many northern fish species possess multiple mtDNA lineages that represent ancestral populations from separate glacial refugia. With the low levels of intraspecific diversity and divergence often observed (Billington and Hebert 1991), it is somewhat remarkable that genetic signatures of multiple refugia can be detected. The modal patterning and discontinuous geographic distribution of intraspecific divergence in many of these species highlight the tremendous demographic and evolutionary impacts that Pleistocene glaciations have had on the genetic structure of northern species.

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Genetic heterogeneity, detected by PCR–SSCP, among samples of larval Pacific oysters (*Crassostrea gigas*) supports the hypothesis of large variance in reproductive success

Gang Li and Dennis Hedgecock

Abstract: Differences in genetic composition among samples of larvae produced during a single spawning season by a semi-isolated population of Pacific oysters (*Crassostrea gigas*) in Dabob Bay, Washington, confirm a specific prediction of the hypothesis that this and other marine animals have large variances in reproductive success. To study the genetics of single larvae, we cloned and sequenced part of the mitochondrial genome and developed polymerase chain reaction (PCR) primers to amplify four segments totaling nearly 2300 base pairs, or 13% of the genome. PCR products were digested with restriction enzymes into smaller fragments, which were then screened for single-strand conformational polymorphisms (SSCP). Seven plankton samples (total $N = 877$), taken between 10 and 21 August 1993, showed a common composite PCR–SSCP haplotype that comprised from 53 to 85% of samples. Nevertheless, exact probability and permutation tests reveal that early and late samples from north Dabob Bay differed significantly from the rest. These differences cannot be ascribed to spatial variation and are consistent with the hypothesis that larvae are produced by relatively few adults, in accord with previous observations of substantial genetic drift in this large population.

Résumé : Les différences dans la composition génétique d'échantillons de larves produites au cours d'une seule saison de reproduction unique par une population semi-isolée d'huîtres creuses du Pacifique (*Crassostrea gigas*) dans la baie Dabob, dans l'État de Washington, confirment de façon spécifique un aspect de l'hypothèse suivant laquelle cette espèce et d'autres animaux marins montrent de grandes variances dans leur succès de reproduction. Pour étudier la génétique des larves, nous avons cloné et séquencé une partie du génome mitochondrial et créé des amorces de PCR pour amplifier quatre segments totalisant près de 2300 paires de bases, ou 13% du génome. Les produits de PCR ont été réduits en petits fragments par digestion avec des enzymes de restriction, fragments que nous avons ensuite criblés pour trouver des polymorphismes de conformation simple brin (SSCP). Sept échantillons de plancton (N total = 877), prélevés entre le 10 et le 21 août 1993, ont montré un haplotype PCR–SSCP composite commun qui correspondait à 53 à 85 % des échantillons. Néanmoins, des tests exacts de probabilité et de permutation montrent que les premiers et les derniers échantillons prélevés dans la partie nord de la baie Dabob différaient significativement du reste. Ces différences ne peuvent être attribuées à la variation spatiale et vont dans le sens de l'hypothèse que les larves sont produites par un nombre relativement restreint d'adultes, comme en témoignent les observations antérieures indiquant l'existence d'une dérive génétique substantielle dans cette grande population.
[Traduit par la Rédaction]

Introduction

Many marine animals occupy vast geographic ranges and often comprise tens of millions to billions of individuals. Yet, these populations generally have only small fractions of the genetic diversity expected on the basis of their sheer abundance (Hedgecock et al. 1982; Nei and Graur 1984; Avise et al. 1988; Avise 1994) and, despite widely dispersing planktonic larvae, often show "chaotic" genetic heterogeneity on small spatial scales (Johnson and Black 1984; Watts et al. 1990; Hedgecock 1994*a*; Edmands et al. 1996; David et al. 1997). To explain both of these paradoxical observations, Hedgecock (Hedgecock et al. 1982; Hedgecock 1994*b*) hypothesized that marine animals in general have extremely large variance in individual reproductive success, owing to high fecundity and sweepstakes-chance matching of reproductive activity with oceanographic conditions permitting maturation, fertilization, larval development, and recruitment.

Two testable predictions flow from this hypothesis. First, by reducing effective population sizes, large variance in reproductive success should make random genetic drift detectable in marine animal populations. This prediction has been confirmed in the few temporal genetic studies that have been made of natural and artificially propagated populations of several marine animals (see Hedgecock 1994*b*). In particular, a case study of a semi-isolated population of the Pacific oyster (*Crassostrea gigas*) in Dabob Bay, Washington, showed that temporal change in allozyme frequencies implied an effective population size of only about 400, perhaps 10 000 times less than the number of oysters harvested from this bay per year (Hedgecock 1994*b*).

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A second prediction of the hypothesis is that there should be detectable genetic heterogeneity among cohorts of larvae if a small proportion of adults produces these. We designed a study to test this prediction, again using the semi-isolated population of Pacific oysters in Dabob Bay. Differential survival of larval cohorts in this model system is governed by wind events that mix warm surface water with deeper colder water, resulting in nearly complete reproductive failure in 3 of every 10 years on average (Schaefer 1938; Westley 1956; Packer 1980; Packer and Mathews 1980). Demonstration of genetic variation among larval cohorts would thus provide a direct link between the recruitment ecology of this population and the genetic drift previously observed.

If one frames this prediction solely in terms of the contributions of females releasing broods into the plankton, then polymorphisms in mitochondrial DNA (mtDNA), a maternally inherited genome, ought to be ideal genetic markers. Enzymatic amplification of DNA by the polymerase chain reaction (PCR) enables genetic study of microscopic organisms and has been successfully used for species identification of larvae for several marine invertebrates (e.g., Olson et al. 1991) and more recently for intraspecific genetic studies (e.g., Côrte-Real et al. 1994). However, large sample sizes are needed to detect the small genetic differences expected among larval cohorts, so a simple method for detecting variation in mtDNA sequences is required.

Electrophoretic separation of single-strand conformational polymorphisms (SSCP) of PCR products (PCR–SSCP analysis) is an efficient method of detecting single base-pair substitutions in DNA (Orita et al. 1989; Cotton 1993; Aguadé et al. 1994). We developed PCR–SSCP based methods for this study by cloning and sequencing part of the mitochondrial genome of Pacific oyster and designing PCR primers for three mtDNA segments totaling nearly 2000 nucleotide base-pairs. In addition, we assayed SSCP variation in a portion of the mitochondrial 16S rRNA gene, using PCR primers previously developed by Banks et al. (1993).

We report here the results of PCR–SSCP analyses of 877 Pacific oyster larvae collected in August 1993 from Dabob Bay, Washington. We compare levels of polymorphism for different segments of mtDNA and test heterogeneity among larval samples in the frequencies of SSCP variants, for both individual mtDNA fragments and composite haplotypes. A concordant pattern of temporal variation is obtained in all analyses, confirming a key prediction of the hypothesis: larvae produced at different times within this bay differ slightly in genetic composition.

Materials and methods

Larval samples

Plankton tows were made every 3 or 4 days in Dabob Bay, Washington, from late July until early September 1993, according to protocols described by Packer (1980) and Packer and Mathews (1980) (see map in Hedgecock 1994*b*). Plankton were killed immediately upon collection with 3% formalin and then transferred into 70% ethanol. Few bivalve larvae were collected before 10 August (cruise C4) or after 21 August (cruise C7). Samples of oyster larvae sufficient for genetic analysis were obtained only from Quilcene Bay (tows from station Q north, labeled by cruise, C5Q, C6Q, and C7Q) and from north Dabob Bay (tows from station 2 to station 1, labeled C412, C512, C612, and C712). Sizes of these samples and distributions of shell lengths are shown in Fig. 1. Plankton samples from the main portion of Dabob Bay (station 7 to 2) contained either too few larvae for population analysis or larvae from which DNA could not be amplified either because of poor fixation or because they were not oysters. The larvae of several other bivalves are found in these plankton samples. Some of them, smaller than 90 μm in shell height, were not distinguishable from oyster larvae by morphology but have been identified by sequences of 16S rDNA amplified by means of universal primers (Kocher et al. 1989; G. Li and D. Hedgecock, unpublished data). None of them provides matching template for the oyster-specific PCR primers described below.

For mtDNA analysis, individual larvae were picked up while being viewed under a dissecting microscope and put into 0.5-mL Eppendorf tubes. Shell height (SH) was measured from the umbo to the growing ventral shell margin. For comparison with the data of Packer and Mathews (1980), SH was converted to shell length (SL) by means of the regression, $SL = 1.143 \times SH - 0.486$, determined from a subsample of 165 larvae ($r^2 = 0.994$). After measurement, larvae were dried under vacuum to evaporate any remaining alcohol. Digestion of larvae was carried out with 10 μL proteinase K digestion solution (1 mg proteinase K and 5 μL Tween 20 in 1 mL of PCR buffer) for 180 min at 56°C, followed by a 15-min treatment at 95°C to denature the proteinase K.

MtDNA extraction, cloning, and sequencing

Adult Pacific oysters, which were spawned in controlled crosses at the Bodega Marine Laboratory and raised in Tomales Bay, California, were used as the source of mtDNA for cloning. The "sucrose mini-gradient" method of Boom et al. (1994) was used to extract mtDNA from approximately 1–2 g of fresh adductor muscle from individual oysters. Extracted mtDNA was digested into five fragments by *Eco*RI and *Xba*I (Oohara and Mori 1989), and ligated into Bluescript SK$^+$ phagemid. The inserts were partially sequenced manually by standard Sanger dideoxy chain-termination methods, according to manufacturer's protocol (Sequenase System, U.S. Biochemical). The WordSearch and Segments programs of the GCG package (Program Manual for the Wisconsin Package, version 7, September 1994, Genetics Computational Group, 575 Science Drive, Madison, WI 53711, U.S.A.) were used to identify similar sequences in the GenEMBL data base.

PCR primers and conditions

Sets of PCR primers were designed, from partial clone-sequences, for three mtDNA segments, designated 5, 8, and 11 (Table 1, Fig. 2). In addition, we used primers previously described for a portion of the gene encoding 16S rRNA (Banks et al. 1993). Optimal PCR conditions (20 μL volumes with 1.0 μL of proteinase K digestion as template) were determined to be 1× PCR buffer (Promega); 0.8 μL of 25 mM $MgCl_2$ for segments 5 and 8, 1.0 μL for segment 11 or 1.2 μL for 16S rDNA; 0.6 μL of 10 mM dNTP for segments 5 and 8, 1.0 μL for segment 11 or 0.16 μL for 16S rDNA; 1.0 μL of 10 μM primer solution; and 0.8 unit of *Taq* polymerase (Promega). PCRs were performed for one cycle of 3 min at 94°C, 2 min at 52°C (50°C for segment 11), and 2 min at 72°C and 29 cycles of 1 min at 94°C, 2 min at 52°C (50°C for segment 11), and 2 min at 72°C with the extension phase of each cycle increasing by 2 s per cycle.

SSCP analysis and scoring of phenotypes

To increase the sensitivity of variation detection, PCR products were enzymatically digested into smaller fragments more suitable for SSCP analysis. Segments 5 and 8 were each digested by *Mfe*I into two fragments, and segment 11 was digested by *Hin*dIII into three fragments, according to manufacturer's protocols. These seven fragments are designated by a combination of segment number and fragment size in base pairs (Table 1) as 5–246, 5–216, 8–279, 8–245, 11–407, 11–322, and 11–247. The 16S rDNA product, which was used whole for SSCP analysis, is here designated 16S-319 or simply 16S.

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Fig. 1. Distributions of shell lengths in samples of Pacific oyster larvae from Quilcene Bay (A) and north Dabob Bay (B) collected in August 1993. *N*, sample size.

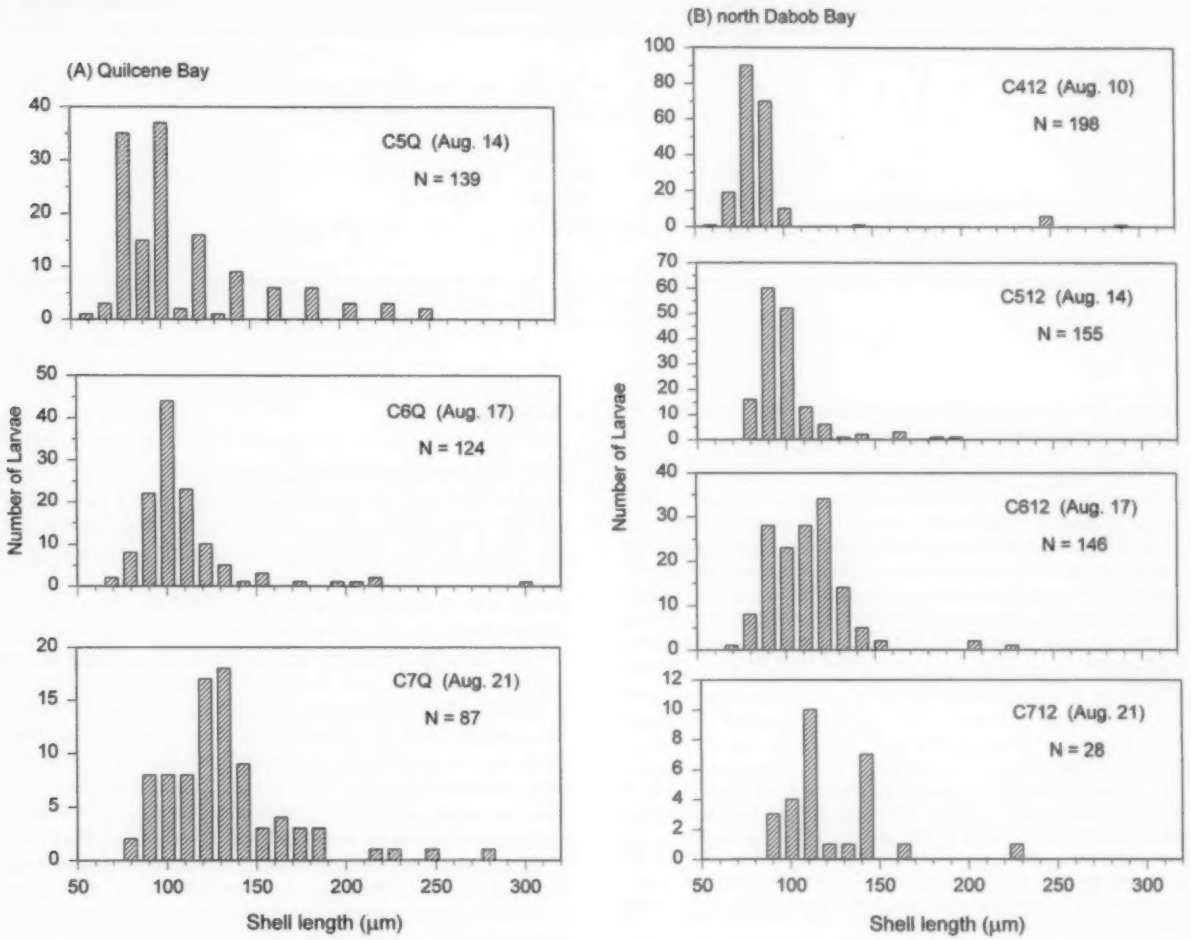

Table 1. Primers and the sizes of PCR products and restriction fragments used in this study.

Primer set	Oligonucleotide sequence	PCR product (bp)	Restriction fragment (bp)
5	+5′-TAAGGATGAG AGCGTTGAGC-3′		
	–5′-TTTCGGGAGG TGCTAATACA-3′	462	246, 216
8	+5′-TTTAGAGTCC CATTTCATCC-3′		
	–5′-ACCACTCTTT CCTCTTCATC-3′	524	279, 245
11	+5′-TACTTTACTT CTCCCCTATC-3′		
	–5′-AGGAGCATAA TAAAGTAAAT AT-3′	976	407, 322, 247
16SA[a]	+5′-CCGGCCGCCC TAGCGTGAGG G -3′		
16SB[a]	–5′-ATTAGCCTGT TATCCCCGGC G -3′	319	—

[a]Data are from Banks et al. (1993).

SSCP sample preparation, electrophoresis, and silver staining were carried out under the same conditions as reported by Aguadé et al. (1994) with minor modification: 0.4× MDE (Mutation Detection Enhancement, FMC BioProducts) gels were run for 16 h at 8 W, and images of silver-stained gels were copied on Automatic Processor Compatible (APC) Duplicating Film (Promega). Restriction fragments for segments 5 and 8 were combined for each individual prior to electrophoresis, while the restriction fragments for segment 11 were combined with the 16S-319 product for each individual for co-electrophoresis in a second MDE gel. Thus, up to 86 individual

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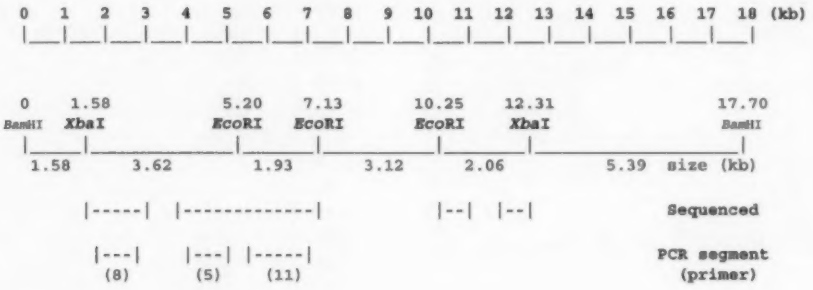
Fig. 2. Diagram illustrating mtDNA segments of *Crassostrea gigas* used for PCR–SSCP analysis (adopted from Oohara and Mori 1989). The whole genome of mtDNA is represented as a straight line, linearized by a single *Bam*HI site. Each restriction site is shown with distance from the *Bam*HI site in kilobases (kb). The three fragments of size 3.62, 1.93, and 2.06 kb were cloned into Bluescript SK+ phagemid; locations of the portions of these clones sequenced and of the segments (5, 8, and 11) for which sets of PCR primers were designed are indicated by dotted lines.



samples plus controls could be simultaneously screened for SSCP for nearly 2300 base pairs (bp), using just two MDE gels.

Mobility shifts in either or both of the single-strand bands corresponding to each fragment were measured and scored as SSCP variants. The measurements were sorted into phenotypic classes, and each class was assigned a letter code, with "A" corresponding to the most common type observed for a given fragment. A letter-coded SSCP phenotype was recorded separately for each fragment successfully amplified and typed from each individual. The order of fragments in composite phenotypes was as follows: 8–279, 8–245, 5–246, 5–216, 11–407, 11–322, 11–247, and 16S-319. Larvae that failed to yield PCR products for two of the four reactions were dropped from the data set.

Statistical analyses

We first analyzed the raw data for each of the eight fragments as if these were independent markers. However, because fragments might not be independent, owing to the absence of recombination in the mitochondrial genome, we determined and analyzed the frequencies of composite haplotypes corresponding both to the complete, eight-fragment SSCP phenotype and to the four-fragment phenotype for the more polymorphic segments 5 and 8.

Data analyses were carried out using the population genetics software packages GENEPOP (Raymond and Rousset 1995) and GENETIX (Belkhir et al. 1997; http://www.univ-montp2.fr/~genetix/genetix.htm). GENEPOP was used for exact tests of homogeneity among larval samples (option 3-1) or between all pairs of populations (option 3-2), as well as for linkage equilibrium (option 2). Settings for dememorization numbers, numbers of batches, and numbers of iterations were adjusted in each case so that the confidence interval for the estimated probability did not include the 0.05 significance level. The permutation test features of GENETIX were used to assess the significance of R_{ij}, the coefficient of correlation between alleles at two loci, as well as the significance of Wright's standardized measure of allele-frequency variance, F_{ST}. The number of permutations was initially set to 500, yielding a 95% confidence interval for the 0.05 significance level of 0.031–0.069, using the normal approximation. When the proportion of permutations yielding as large or larger a parameter as that observed fell within this interval or was 0.000, the test was rerun with 5000 permutations (95% confidence interval, 0.044–0.056). Sets of samples having homogeneous haplotype frequencies were determined by cumulative stepwise removal of individual plankton-tow samples until a nonsignificant F_{ST} was obtained for the remaining samples; the order of sample removal was based on the amount of reduction in the F_{ST} after removal of each population and the significance of F_{ST} for the remaining samples.

Results

PCR amplification of mtDNA segments

Three of the five restriction fragments obtained by digestion of isolated mitochondrial DNA with *Eco*RI and *Xba*I are cloned into phagemid (Fig. 2). A total of about 4000 nucleotide bp is known from sequencing the ends of these three clones, but design of PCR primers for amplifying any of this DNA is made difficult by high A:T content (~65%). Of the two dozen sets of primers initially designed, only seven work on crudely extracted mtDNA, and only three of these can be routinely used to amplify DNA successfully from proteinase K digested larvae. Together with the 16S rDNA, a total of four mtDNA segments can be amplified from single larvae for SSCP analysis; these fragments range in size from 319 to 976 bp and total 2281 bp, about 12.9% of mitochondrial genome of the Pacific oyster (Oohara and Mori 1989; Boom et al. 1994). Fragment by fragment, the overall percentages of single-larva digestions that yielded PCR–SSCP results range from lows of 72–74% for the three fragments of the longest segment, 11, to 98% for the two fragments of segment 8 (Table 2).

The complete nucleotide sequences of segments 5, 8, and 11 have been determined (GenBank accession nos. AF034688, AF034689, and AF034796) and analyzed for homology with other sequences in the GenEMBL data base. Segment 8 shows high homology to a 524-bp segment of cytochrome *b* (corresponding to amino acid residues 217–378 of the *Drosophila* protein); the sequence of this clone will be presented elsewhere, together with a DNA-sequence analyses of its SSCP variants (G. Li and D. Hedgecock, unpublished data). Results for segments 5 and 11 are much less clear. When the nucleotide sequences for segments 5 and 11 (Fig. 3) are translated into amino acid sequences, using the invertebrate mitochondrial code (SGC4; National Center for Biotechnology Information), there are 6–15 termination codons in segment 5 and 7–23 stops in segment 11. The nucleotide sequence for segment 5 is A+T-rich and is thus somewhat similar to the origin of heavy-strand replication in the *Drosophila melanogaster* mitochondrial genome, but regions of comparison are short, and overall nucleotide identity is low. On the other hand, portions of the minus strand of segment 11, particularly between nucleotides 42–150, at the start of fragment 11–322, and

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Table 2. Genetic variation and diversity as detected by PCR–SSCP analysis of eight mtDNA fragments in 877 larvae of *Crassostrea gigas*.

Fragment	No. larvae assayed by PCR (%)	No. SSCP variant classes	No. variants (%)	F_{ST} among larval samples	Percent permutations $\geq F_{ST}$[a]	Exact probability test
5–246	679 (77.4)	8	22 (3.2)	–0.0038	84.2	0.161
5–216	680 (77.5)	14	38 (5.6)	0.0206	0.2	0.000***
8–279	857 (97.7)	25	71 (8.3)	0.0042	10.8	0.165
8–245	857 (97.7)	18	45 (5.3)	0.0126	0.2	0.200
11–407	639 (72.9)	14	34 (5.3)	0.0015	27.6	0.718
11–322	629 (71.7)	5	11 (1.7)	0.0183	1.2	0.029*
11–247	652 (74.3)	1	5 (0.8)	–0.0062	88.0	0.945
16S–319	831 (94.8)	4	7 (0.8)	–0.0054	100.0	0.781
Total	—	89	—	0.0076	0.02[b]	0.005**

[a]Percentage of 500 permutations of data that give an F_{ST} as large or larger than the observed.
[b]Percentage of 5000 permutations of data that give an F_{ST} as large or larger than the observed.
*$P < 0.05$.
**$P = 0.01$.
***$P = 0.001$.

383–461, spanning much of fragment 11–247, have 60–90% nucleotide identity with many 12S rDNA sequences, including those of the scallop (*Pecten maximus*) and the seastars (*Henricia napponica* and *H. pachyderma*). No convincing homologies were observed for the sequence corresponding to fragment 11–407 however. Thus, the identities of segment 5 and about half of segment 11 remain uncertain.

Variation detected by PCR–SSCP and linkage disequilibrium

For each of the mtDNA fragments, the majority of individuals analyzed by PCR–SSCP methods shares a common SSCP phenotype. The frequency of this common phenotype varies from 0.992 for two rDNA fragments, 16S-319 and 11–247, to 0.917 for the cytochrome *b* fragment 8–279. The combined frequencies of variants for each of the fragments thus varies by a factor of 10, from 0.8 to 8.3% (Table 2). The number of variant SSCP phenotypes or classes, each detected by shifts in the electrophoretic mobility of one or both strands of a fragment relative to the positions of the common single-stranded molecules, ranges from 1 for fragment 11–247 to 25 for fragment 8–279, with a total over all eight fragments of 89 (Table 2). The total number of haplotypes thus revealed by PCR–SSCP in our samples of Pacific oyster larvae from Dabob Bay is 97.

When linkage disequilibria for a total of 196 pairwise comparisons of fragments in seven larval samples are tested by the estimated exact probability method, there are only seven (3.6%) rejections of equilibrium, involving five fragments and six of seven larval samples (Table 3). Six of the seven rejections involve fragment 5–216. However, these significant results are in each case attributable to just two individuals (three individuals for fragment pair 5–216 and 8–279 in C412) carrying rare types at the two fragments compared. The correlation coefficients associated with these seven significant linkage disequilibria range from 0.061 to 0.881, and the significance of the five largest are supported by permutation tests (Table 3). In the total population, the two fragments of segment 5 are found to have a weak linkage disequilibrium (with 3.84% of 5000 permuted values equaling or exceeding the observed correlation, $R_{ij} = 0.021$, a percentage below the lower 95% confidence limit for $P = 0.05$).

Both composite haplotypes, the first corresponding to the four-fragment SSCP phenotype for segments 5 and 8 and the second, to the complete eight-fragment SSCP phenotype, show the same pattern of diversity as seen in each of the separate fragments, a common haplotype shared by a majority of the population with many rare haplotypes. For the four-fragment composite scoring, the common "AAAA" haplotype comprises from 68 to 89% of the individuals within a sample; the number of haplotypes per sample ranges from 6 to 23. Of the total of 68 haplotypes observed over all samples ($N = 666$), 49 (72%) are found in only a single individual. For the eight-fragment composite scoring, the common "AAAAAAAA" haplotype comprises from 53 to 85% of the individuals within samples; the number of haplotypes per sample ranges from 7 to 23. Again, of the total of 72 eight-fragment composite haplotypes observed over all samples ($N = 436$), 53 (73.6%) are found in only a single individual.

Heterogeneity among samples

Genetic differentiation among the seven larval samples, on a fragment by fragment basis (Table 2), is highly significant by permutation test for fragments 5–216 and 8–245, significant for fragment 11–322, and not significant for the five remaining fragments (5–246, 8–279, 11–247, 11–407 and 16S-319). Exact probabilities for the same data show similar significance levels, except for a nonsignificant result for fragment 8–245. Over all eight fragments, the combined F_{ST} of 0.0076 is highly significant, with only one of 5000 (0.0002) permutations of the population data equaling or exceeding this value and a combined exact probability of 0.0054 (Table 2). Stepwise sample removal shows that sample C712 yields the greatest reduction in F_{ST}, but the remaining samples are still significantly heterogeneous (Table 4A). Removal, next, of either samples C412 or C612 leaves the remaining five populations homogeneous but with nearly significant F_{ST}s of 0.0015 ($P = 0.118$) and 0.0028 ($P = 0.059$), respectively (where P is the proportion of permutations producing values equal to or greater than the observed). If all three samples, C712, C612, and C412, are removed, F_{ST} for the remaining four samples drops to –0.0014 ($P = 0.720$). The stepwise removal procedure shows that the three north Dabob Bay samples are themselves heterogeneous; removal of

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Fig. 3. Sequence of two segments of mtDNA cloned from *Crassostrea gigas*. Underlined nucleotides correspond to primers. (A) A 462-bp sequence of segment 5. Restriction site of *Mfe*I is given in boldface. (B) A 976-bp sequence of segment 11. Restriction sites of *Hind*III are given in boldface.

(A)

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TAAGGATGAG AGCGTTGAGC TTTTAACTCA AATGTGAAAC TAGTGTTTTC   50
GATTCTAAAA AATAATTTTG CCCTAAGCTT TCATAAAGTA GTTTAATGCA  100
AAACGTGAAA TTGCAAATTT TAAGATGCCC AAGAAATTGG CCTTTATGTC  150
TAAAAAATTA GCTTCATGAG AATACTGGTG TTGGCTTAGG CTTAAATTAT  200
TAAAGTTAAT TCAGCAATTG GATAAATGTA TTTGAAACAT TAATTTTTAG  250
CAAAAGCTAG GCTTTAAGTA TACCAAAGGT ATGGGGACTA CTAGCAATAA  300
ATAGCAGTTT AAAAACTTTT TCTAAATGAA GTATAGGAGC ATTAGAAACA  350
TTATTTTAGA TTTTGTCTGG TAAGATGAAA GCATTGTTTT TAAGCGTTAA  400
AAAAAAGTAC CTTTAGTATA ATGGCCTTTC AAGAGATTTT GCTGTATTAG  450
CACCTCCCGA AA
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(B)

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TACTTTACTT CTCCCCTATC ATTAAAAGAG CAAGTTCCCT ACCTTACCAT   50
GTTACGACTT ACCTCTCCTT TCGGCTAGGG TGACGGGCGA TTTGAACGCA  100
CCGCAGTTGC GTCTTTTCAA AAAAACGAGT TACTCTCTTT TTACTCCCAA  150
ATCCTTATAT ACACTAAGAG AATTTCATCC CTAAAATCTT TATATGTCTA  200
AATATTTGTC TCTCAGCTAA CCCCTTGCCC ATAAGGAGCA CTTCGACCTG  250
ACCTAGCCTC GGCATGATTA TTAATTACTT AAAACCCCAA GATTGGGCCT  300
ATTTTTATTT ACTCACATCA TAAGCTTTCG ACGGCAGTAC ACTGCTTTCT  350
ATAGGGCAGG TAAGATATCA CGCGGATCAT CAGATTAAGC GCCAAGAGCC  400
TCTGGATGGT TATGCGGAAC CGCCAAGTTC TTCGAGTTTT AAGCAGTTGC  450
TCGTAGTACC CCTAGCAAAC TATTAATTCA AATAAAATAG CTATACTTGA  500
CATTATGGAA TAACCGGGTA CCAAATCCGG GTTCTATGCC CATAAACTTC  550
GCAGATTCAT CTTTACGCAA GCTTTGGACT CCCGCAAGAC TGAGATTTCA  600
CCCATAGTCC TTGCTGTATG TCTTGCGTCA CACATTTTTG ACTAACTGCC  650
TCTAAGCTGC ACCCTTATTA GCTCCTCGAG AGAGGGAGTG ACCGCAGCTG  700
CTGGCACCTC TTTCGCTTCG ATTTAGTTAG TTTTTTATTC GTTTCAAGCT  750
AGAACTCATT GATTTCCAGA TCTACCCACT TGAGTTGTGA TTTCCTTCGC  800
GTCATCATTA AAAACTTATG CTCCTGCTGC CAGCTATTCC TTTTAGGATG  850
CATTAGACTT GGCTTATCAC GTAAATCAGA CTTTTACACA TCACGGAATT  900
ATCCTACATT GTGTAATGTA AAAAACTCTG TAGCTAACAT TATACCAGCA  950
CCAAATATTT ACTTTATTAT GCTCCT
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C712 makes the F_{ST} for C412 and C612 nonsignificant (Table 4B).

Results comparable with the above are obtained in analyses of the four- and eight-fragment composite haplotypes (data not shown), despite the smaller numbers of individuals (666 and 436, respectively) from which all constituent segments of the composite haplotype could be successfully amplified. For the four-fragment composite haplotype, F_{ST} over all seven larval samples (0.0146) is larger than that for independent fragments, with permutation and exact probabilities of 0.0012 and zero, respectively. Removal of C712, C612, and C412 is necessary to reduce F_{ST} to a nonsignificant value; the three north Dabob Bay samples are themselves homogeneous. A similar pattern is observed for the eight-fragment composite haplotype, which yields an overall, highly significant F_{ST} of 0.0239.

Discussion

The hypothesis of large variance in reproductive success postulates a strong and direct linkage between population genetics and the recruitment problem in biological oceanography. Events in the early life history stages of marine organisms, especially those species with a highly dispersing pelagic larval phase, are often critical to the dynamics of the adult populations and perhaps also to their genetic structure. However, the inability to obtain genetic information from often-microscopic planktonic larvae has been a long-standing problem in marine biology (Olson et al. 1991). The use of PCR-based techniques opens up a wide range of possibilities for population genetic study of relatedness within or differences among larval cohorts (Hu and Foltz 1996; Côrte-Real et al.1994; Ruzzante et al. 1996; Herbinger et al. 1997). The particular PCR-based method that we have used here, PCR–SSCP of mtDNA, appears generally promising for evolutionary biology (Orti et al. 1997; Bagley et al. 1997), including, as shown here, population genetic study of marine planktonic larvae. In particular, testing the hypothesis of large variance in reproductive success is facilitated by the simplicity of PCR–SSCP (Orita et al. 1989). Once suitable primers have been designed, large numbers of individuals can be assayed quickly and with minimal expense, matching the speed and cost effectiveness of allozyme assays. Sequencing of the partial cytochrome *b* gene amplified by primer 8 from 104 selected individuals shows that the efficiency of variation detection of our PCR–SSCP procedure is about 90% (G. Li and D. Hedgecock, unpublished data).

The nature and extent of mtDNA variation

The eight different fragments of mtDNA studied differ substantially in the level of SSCP variation and thus in their utility for detecting differences in the genetic makeup of larval samples. All fragments are represented in the population by a predominant common haplotype, but the summed frequencies for variants at each fragment varies over a 10-fold range, from 0.8 to 8.3% (Table 2). Fragment 16S-319 is nearly fixed, and segment 11 has both a low polymorphic level and a high rate of failure to amplify from single-larva digestions, presumably because of its larger size (Table 2). Thus, these two PCR products have limited application as genetic markers and could be eliminated from future study. Indeed, separate analysis on a composite haplotype comprising only segments 5 and 8 sharpened distinctions among samples. Although the mitochondrial genome does not have recombination, only 4% of 196 tests for linkage disequilibrium are significant. Each of these significant tests is attributable to two, in one case three, individuals within a sample that happen to have rare variants at two fragments. Polymorphic mtDNA fragments in Pacific oyster populations thus behave virtually as independent genetic markers.

Our results on the nature and extent of mtDNA variation in Pacific oysters of Dabob Bay agree in three major respects with RFLP data reported earlier by Boom et al. (1994), who

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Table 3. Tests of linkage disequilibrium between pairs of fragments.

Sample	Fragment pair	Exact probability		Permutation	
		P	SE	R_{ij}	$\geq R_{ij}$
C5Q	5–216, 5–246	0.00478	0.00141	0.457	0.6%
C6Q	5–216, 8–279	0.04235	0.00602	0.094	13.8%
C7Q	5–216, 8–245	0.00165	0.00034	0.881	0.9%
C412	5–216, 8–279	0.00784	0.00232	0.239	0.6%
C412	5–216, 8–245	0.04608	0.00873	0.061	8.4%
C512	5–216, 5–246	0.00684	0.00106	0.196	3.5%[a]
C612	11–407, 8–245	0.00197	0.00029	0.245	0.6%

[a]5000 permutations used.

included 18 individuals from this same population in a study, primarily, of Pacific oysters from British Columbia. First, both studies find a predominant haplotype in the Dabob Bay population, comprising 12 of 18 adults in the Boom et al. sample and from 60 to 90% of the 877 larvae we studied. (In British Columbia populations, Boom et al. found two common haplotypes that together comprise about 60% of adults; these two haplotypes differ from each other by a single *Hin*dIII restriction site.) Second, both studies find that variant haplotypes differ little from the common haplotype(s), in most cases by a single restriction site, a single SSCP class difference, and probably by a single nucleotide substitution (confirmed in the case of SSCP variation by sequencing of cytochrome *b* and 16S variants; G. Li and D. Hedgecock, unpublished data). Thus, mtDNA haplotypes in North American Pacific oyster populations lack the deep phylogenetic divergences often observed in other animals (Avise 1994), suggesting that variants are recently derived by mutation from the common, ancestral sequence (Beckenbach 1994). The absence of strong linkage disequilibrium in our data can also be seen as a consequence of the recent origin of variant haplotypes. Both of the foregoing observations imply that the effective size for this population is small, likely owing to large variance in reproductive success (Beckenbach 1994; Hedgecock 1994*b*) and in agreement with the inference from temporal change in allozyme frequencies (Hedgecock 1994*b*). Finally, both studies find a large number of closely related, variant haplotypes in Pacific oyster populations. Over 70% of haplotypes observed in both studies are singletons, haplotypes found in only a single individual. Such large numbers of rare haplotypes and singletons are unexpected in a small population (Boom et al. 1994), a paradox that Beckenbach (1994) suggests could be explained by higher than usual mutation rates for oyster mtDNA.

Larval production in Dabob Bay in 1993

There was essentially no settlement of Pacific oyster larvae in Dabob Bay in 1993 (Washington State Department of Wildlife and Fisheries, Port Whitney Shellfish Laboratory, Brinnon, Wash.), as is typical of cold, windy years (Schaefer 1938; Westley 1956; Parker 1980; Parker and Mathews 1980). Average bay surface temperature in 1993 was above 20°C, the minimum for oyster spawning, only on 5 August and otherwise remained close to 19°C, barely above the minimum for adequate larval development, from 26 July to 21 August, when it plunged to 15°C. Few larvae were present in plankton tows taken before 10 August or after 21 August. The size-frequency

Table 4. Heterogeneity in the frequencies of eight mtDNA fragments treated as independent markers.

Sample(s) removed	F_{ST}	Proportion of permutations ≥ observed F_{ST}
(A) Effect on F_{ST} of cumulative stepwise removal of samples.		
None	0.0076	0.0002[a]
C712	0.0036	0.010
C412	0.0073	0.004
C612	0.0079	0.000
C712, C412	0.0015	0.118
C712, C612	0.0028	0.059[a]
C712, C412, C612	–0.0014	0.720
(B) Stepwise removal from the set of three north Dabob Bay samples (C712, C412, C612) causing heterogeneity in (A).		
None	0.0138	0.004
C712	0.0029	0.158

[a]5000 permutations used.

distributions of sampled larvae (Fig. 1) show both a steady decline in numbers of early larvae, reflecting a reduction in spawning after the first half of August, and few larvae surviving beyond 130 μm in shell length, well below the settlement size of 310 μm.

The poor production of oyster larvae in Dabob Bay in 1993 has both procedural and interpretive implications for our study. The former arises from the absence of distinct cohorts in the plankton samples, which prevents us from making an explicit comparison of larvae spawned on different days. The 1993 larval size-frequency distributions (Fig. 1) are unimodal, in contrast to those reported by Packer (1980) and Packer and Mathews (1980) for larvae in 1977 and 1978. However, changes in modal larval size over time are inconsistent with the expected rate of growth at 19°C (Packer and Mathews, 1980), suggesting that there was high mortality of early larvae and that larvae sampled on different dates originated from different parents. To the extent that larvae sampled on different days were spawned at the same time, by the same parents, our temporal samples should have been genetically homogeneous. We observe differences among larval samples despite any such bias towards homogeneity.

According to the hypothesis of large variance in reproductive success, we expect variance of individual reproductive contributions to be enhanced precisely when the spatiotemporal windows or opportunities for successful recruitment are narrow. From this point of view, 1993 was an excellent year to have looked for the predicted genetic differences among larval cohorts. On the other hand, mean reproductive success in this year was probably close to zero, so that the demonstrated differences among larval samples would not have produced genetic drift in the population. Further observations, in years when distinct cohorts of larvae are present and, because of interspersed wind events, make differential contributions to settlement, are needed to link directly variation among larval cohorts to genetic drift in recruits. Nevertheless, regardless of the ultimate failure of larvae to recruit in 1993, the demonstration of differences among larval samples supports a key prediction of the hypothesis: larvae at any one time and place are produced by finite numbers of adults giving rise to a sampling

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variance in the haplotype and allelic frequencies among potential recruits.

Temporal heterogeneity among larval samples

The predominance of a common haplotype in all samples makes the statistical analysis of heterogeneity problematic. We used two different approaches, estimation of exact probabilities for allele frequency tables and random permutations of individuals among samples to determine the likelihood of obtaining the observed F_{ST} by chance. In general, the two approaches yield comparable results, but a noteworthy and instructive exception is provided by the heterogeneity of haplotype frequencies for fragment 8–245, which is significant by the permutation test but not significant by the exact probability method. Examining the contributions of individual alleles to total F_{ST} for this fragment shows that virtually all of the among-sample variance can be attributed to variation in the frequency of the common haplotype. If rare alleles are pooled into one class, the exact probability of sample homogeneity of haplotype frequencies for 8–245 becomes a significant 0.0029. Thus, it appears from this case that exact probability tests of large, sparse tables can be overly sensitive to homogeneity of rare alleles and can miss significant variation in the frequency of the most common allele.

Whether we analyze frequency data for individual fragments or for composite haplotypes, we find a consistent pattern of variation among samples. Larvae in Quilcene Bay were homogenous throughout the sampling period, but larvae in north Dabob Bay on the 10 August, 17 August, and particularly 21 August had significantly different haplotype frequencies than those in the remaining samples.

Since the genetic heterogeneity of larval samples arises primarily from plankton tows taken in north Dabob Bay, to what extent might this be interpreted as spatial rather than temporal heterogeneity among spawning populations? There is abundant evidence that neither immigration nor subpopulation structure within Dabob Bay can account for our results. First, historical plankton sampling along a transect from lower to upper Dabob Bay for over 30 years by the Washington State Department of Fisheries shows that immigration of larvae from Hood Canal is rare (Westley 1956; Packer 1980). In 1993, in particular, plankton samples from the lower part of Dabob Bay contained very few or no oyster larvae, ruling out the possibility that immigration of larvae from a different population could explain our results. The unimodal distributions and small sizes of the larvae sampled also attest to their local production. Likewise, there is no reason to suspect that a body of water less than 20 km long and 5 km wide could contain genetically different subpopulations, and accordingly there is no evidence for this, either in allozyme data (D. Hedgecock and D.J. McGoldrick, unpublished data) or in preliminary analyses of SSCP mtDNA data for spat and adults collected in 1994 (D. Hedgecock and G. Li, unpublished data). Of course, a spatial pattern might be present in early larvae, such as we sampled, owing simply to the location of spawning adults; this spatial pattern might have disappeared had larvae continued to develop and to diffuse over larger areas, but high early mortality prevented this. We find it difficult, therefore, to ascribe the variation among larval samples reported here to any source other than variation in adult reproductive contributions.

The enormous fecundity of oysters (a single female *Crassostrea gigas* can spawn on the order of 100 million eggs; Galtsoff 1964) makes it numerically possible for very few of them to replace the entire population in Dabob Bay. We nevertheless expect that, even if variance in reproductive success is large and effective population size is only on the order of 400 (Hedgecock 1994*b*), the total number of females contributing to larval production would make the chances of sampling maternal sibs or half-sibs remote. In light of this expectation and the rarity of SSCP mtDNA variants in this population, individuals sharing the same rare haplotype in our samples attract attention. For example, three individuals with the SSCP haplotype EJ for fragments 5–246 and 5–216 are found, one in the first sample from north Dabob Bay (74 μm SL) and two in the second sample taken there 4 days later (92 and 147 μm SL). Even more striking are the four individuals of haplotype AAAFAAAA found in the last sample from north Dabob Bay and largely responsible for the highly significant divergence of this sample from others. Are such individuals maternal sibs or half-sibs? Microsatellite analysis can now be used to approach such questions (Ruzzante et al. 1996; Herbinger et al. 1997), but PCR–SSCP of mtDNA offers a highly efficient method for prescreening large samples to locate appropriate candidates for more detailed microsatellite analyses of relatedness.

The hypothesis of large variance in reproductive success makes specific predictions that are beginning to be tested in natural populations of marine animals, either by analysis of temporal genetic change (Hedgecock 1994*b*; Purcell et al. 1996) or of relatedness within or differences among larval populations (Ruzzante et al. 1996; Herbinger et al. 1997). These are the first genetic data for invertebrates to support the prediction that larvae sampled at different times should give evidence of having been produced by finite numbers of adults. The results are consistent with the previous observation of genetic drift in the same population (Hedgecock 1994*b*). More sampling of larval stages will be necessary to establish the critical link between variation in individual adult contributions to the pool of planktonic larvae and variation in their contributions to actual recruits. Larger larval samples would allow comparisons of size categories within plankton tow samples, and more frequent samples would allow comparisons among years that may differ dramatically in total larval production and recruitment success. Combining the highly efficient PCR–SSCP method for screening mitochondrial haplotypes with analyses of nuclear microsatellite DNA to determine relatedness is likely to offer new insights into the temporal structure and recruitment ecology of marine animal populations.

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Brown trout (*Salmo trutta*) habitat use and life history in Swedish streams: possible effects of biotic interactions

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Abstract: To test if habitat use and life history of stream-dwelling brown trout (*Salmo trutta*) differed between allopatric and sympatric situations, we compared three streams with differing fish communities and used data from a large national database containing electrofishing results from Swedish streams. In the three-creek study, allopatric brown trout used all habitats and shifted from nursery areas in riffles to pool habitats, where adult growth and survival were higher. Mainly females shifted habitat and this was undertaken after age 1. Sympatric brown trout under intense pressure from other fish species remained in the riffles throughout their life cycle. Under moderate pressure from other species, larger brown trout used slow-flowing habitats. Early growth was more rapid in sympatry. Sympatric brown trout also had a lower adult to juvenile growth ratio and lower adult survival and matured earlier than allopatric brown trout. The data from the nationwide database showed that frequency of occurrence and abundance of brown trout were negatively associated with the number of coexisting fish species. It was also verified that the habitat shifts between riffles and pools were more common and possibly more beneficial in terms of growth and survival in allopatry. In addition the existence of differences in juvenile growth between allopatric and sympatric populations was verified.

Résumé : Pour vérifier si l'utilisation de l'habitat et le cycle vital de truites brunes (*Salmo trutta*) vivant en cours d'eau étaient différents dans des conditions allopatriques et sympatriques, nous avons comparé trois cours d'eau renfermant des communautés de poissons différentes et utilisé des données d'une grande base de données nationale renfermant des résultats de pêche électrique obtenus dans des cours d'eau de la Suède. Dans l'étude sur les trois ruisseaux, les truites brunes allopatriques ont utilisé tous les habitats et sont passées des zones d'alevinage, dans les rapides, à des fosses, habitats où les taux de croissance et de survie des adultes étaient plus élevés. Le changement d'habitat était surtout le fait des femelles et se faisait après l'âge de 1 an. Les truites brunes sympatriques qui subissaient de fortes pressions de la part des autres espèces de poissons sont demeurées dans les rapides durant tout leur cycle vital. Quand la pression issue des autres espèces était modérée, les truites brunes de plus grande taille fréquentaient des habitats à faible courant. La croissance en bas âge était plus rapide dans les conditions sympatriques. Les truites brunes sympatriques montraient aussi un plus faible rapport entre le taux de croissance des adultes et le taux de croissance des juvéniles et un plus faible taux de survie des adultes, et elles arrivaient à maturité plus rapidement que les truites brunes allopatriques. Les données de la base de données nationale ont montré que la fréquence d'occurrence et l'abondance de la truite brune diminuaient avec le nombre d'espèces de poisson coexistantes. On a aussi observé que le passage des rapides aux fosses était plus fréquent et peut-être plus bénéfique au chapitre de la croissance et de la survie dans les conditions allopatriques. Enfin, on a pu vérifier l'existence de différences dans la croissance des juvéniles entre les populations allopatriques et sympatriques.
[Traduit par la Rédaction]

Introduction

On a large geographic scale, predation and interspecific competition affect distribution patterns of individual fish species (Degerman and Sers 1992, 1993; Gilliam et al. 1993; Fraser et al. 1995) whereas habitat use is influenced on a smaller scale, i.e., within the actual stream (Power 1987; Brown and Moyle 1991). Experimental studies have also revealed effects on life history parameters such as growth rate and survival (Fraser and Gilliam 1992). In populations where individuals range widely in size, the habitat use of an individual will vary depending on life stage or size (Werner and Gilliam 1984). Size-specific habitat shifts during ontogeny often enhance survival and (or) growth. Consequently, habitat shifts often influence factors associated with growth such as fecundity as well as age and size at maturity (Werner 1988). Size-dependent ontogenetic habitat shifts in size-structured populations can be strongly affected by biotic interactions such as interspecific competition and predation (Werner and Hall 1988; Persson and Eklöv 1995).

Salmonid fish species grow continuously throughout their life span, which is a typical characteristic of size-structured populations. They are flexible in terms of being able to cope with different environments. As a consequence, these species provide examples of different life histories in response to variation in environmental factors. In studies of life history variation in stream salmonids, the physical environment and its

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Table 1. Drainage area, height above sea level, gradient, and annual mean flow for the study areas in the respective creeks.

	Bjuråsbäcken	Storbäcken	Låktabäcken
Drainage area (km^2)	9.7	54	75
Height above sea level (m)	329–410	37–52	342–422
Gradient (%)	2.7	0.76	2.8
Annual mean flow (m^3/s)	0.087	0.50	1.6
Riffle mean depth (m)	0.23 (0.01)	0.21 (0.01)	0.32 (0.02)
Pool mean depth (m)	0.55 (0.01)	0.36 (0.01)	1.57 (0.09)
Riffle mean width (m)	2.6 (0.1)	4.5 (0.2)	7.1 (0.3)
Pool mean width (m)	2.6 (0.1)	4.5 (0.5)	15.3 (0.8)
Length of study area (m)	3100	1500	2900
% pools of study area	18	70	13

Note: Mean depth and width (SE given in parentheses) for the riffle and pool habitats and percentage pool habitat are given for each study area.

effect on growth rate have been the main subject. In most cases, allopatric populations or situations where the influence of other fish species have been negligible have been examined. Often, large-scale comparisons of interpopulational variation have been undertaken, with effects of latitude being the main theme (e.g., Withler 1966; L'Abée-Lund et al. 1989; Metcalfe and Thorpe 1990; Jonsson and L'Abée-Lund 1993). Thus, climate and its effect on water temperature and, consequently, on fish growth have been pointed out as a key to understanding variation in life history between populations. However, considerable differences in life history may also exist between populations within the same geographic region and in similar habitats. Hutchings (1993) studied life history in stream-resident populations of brook trout (*Salvelinus fontinalis*) and concluded that they were adapted to their local environment on a very small geographic scale. Again, populational differences in growth rate seemed to be a key factor explaining the observed life history variation in line with life history theory (cf. Roff 1984; Stearns and Koella 1986). In Hutchings' (1993) study, predation and competition from other fish species were nonexistent, which emphasised the importance of differences in prevailing environmental conditions in explaining variation in life history. However, in more complex situations with several species coexisting, predation and interspecific competition may contribute substantially to shaping the life history of stream-dwelling salmonid populations. Brown trout (*Salmo trutta*) is a very flexible species in terms of life history and habitat use (Jonsson 1985; Elliott 1994). In Sweden, sea-run, lake-run, lake-resident, and stream-resident populations occur. Stream-resident populations are very common, both allopatric ones and populations sympatric with several other species. Given the information from other species and from previous studies (Degerman and Sers 1992, 1993), we would expect differences in habitat use between stream-resident populations depending on the presence of predatory and competing fish species. We predict that (*i*) in presence of other fish species (sympatry), less habitat types would be utilised than in allopatric situations and (*ii*) as a consequence of the constraints in available habitats, adult growth and survival would be lower in sympatric than in allopatric brown trout. To test these expectations, we compared three streams, all with stream-resident brown trout but with different fish communities. In addition, we used data from a national database containing electrofishing results from Swedish streams to verify data on habitat use and early growth. In this way, data from a wide range of localities were tested for effects of species composition on brown trout habitat use and life history traits.

Table 2. Species composition (% in weight) in the various habitats in the study streams as revealed by electrofishing.

	Bjuråsbäcken		Storbäcken		Låktabäcken	
Species	Riffles	Pools	Riffles	Pools	Riffles	Pools
Brown trout	100	100	46	58	96	0
Grayling	0	0	0	0	1	60
Yellow perch	0	0	0	0	0	17
Northern pike	0	0	0	22	1	18
Burbot	0	0	1	0	1	5
Minnow	0	0	0	0	1	+
Bullhead	0	0	53	20	0	0

Note: Data from pool habitats in Låktabäcken are gillnet catches. +, minnows were observed but not captured.

Material and methods

Three-creek study

Study areas

Bjuråsbäcken (63°03′N, 15°72′E) is a tributary of Sittån Creek which in turn empties into the River Indalsälven in central Sweden. It is the smallest of the three creeks studied (Table 1); it originates in a peat-bog area and has no lakes within its catchment. Brown trout is the only fish species present. Bjuråsbäcken is steep in the lowermost section which prevents fish (including brown trout) from entering the creek from downstream areas. Storbäcken (64°10′N, 20°50′E) is located in the northeastern part of Sweden and is a 28-km-long tributary to the River Dalkarlsån which flows into the Gulf of Bothnia. It flows through a mixture of woodlands and agricultural land. Storbäcken holds resident brown trout. No immigration of large-sized spawners from downstream areas has been observed during electrofishing during the spawning period. Storbäcken also supports populations of northern pike (*Esox lucius*), burbot (*Lota lota*), bullhead (*Cottus gobio*), and lampreys (*Lampetra* spp.) (Table 2). Låktabäcken (65°72′N, 16°93′E) is a tributary to the River Vindelälven in northern Sweden. It has a steep gradient and a drainage area of 75 km^2 (Table 1). The creek has several lakes within the drainage area, of which Lake N. Låktaträsket is located at the upstream end of the study section. A fish trap at the mouth of the creek has shown that brown trout, the dominating fish species, is stream resident (Näslund 1993). Låktabäcken also holds grayling (*Thymallus thymallus*), minnow (*Phoxinus phoxinus*), yellow perch (*Perca fluviatilis*), northern pike, and burbot (Näslund 1989).

Habitat

To characterise the physical habitat, measurements were made of stream width, stream depth, and the surface area of boulders along transects every fifth mere of linear length along the sections surveyed by electrofishing. The study sections of Bjuråsbäcken, Storbäcken, and Låktabäcken were divided into two distinct types of habitat (Table 1). In all creeks, slow-flowing, "flat" sections are characterised by finer substrate (silt–sand) and a greater mean depth compared with the riffle sections (Table 1). To simplify the habitat description and treatment of the data, all such habitat will be characterised as pool habitat. In Bjuråsbäcken, pool habitats are distributed over a 100-m-long section in the lower part, five short (15–25 m) sections in the middle part, and a 350-m-long section in the uppermost part of the creek. In Låktabäcken, there were two pool sections situated in the

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middle part of the study section, one being about 320 m long and the other about 40 m long. The study area in Storbäcken is dominated by pool habitat. Long (30–200 m) flat sections are separated by short riffle sections.

Methods

Electrofishing (three successive removals) was performed in the riffle habitat of each stream. The electrofishing sampling in Storbäcken was performed using a 400- to 600-V, pulsed DC apparatus (BIOWAVE II ver. 2.05). In Bjuråsbäcken and Låktabäcken, an apparatus providing a continuous DC of 600–800 V (LUGAB 1000s) was used. Fourteen sections in Låktabäcken were fished during August 15–25, 1983, four sections in Bjuråsbäcken were fished in August 1994, and five sections in Storbäcken were sampled from August 29 to September 5, 1995. Population estimates for these sections were calculated according to Bohlin (1981). Fish biomass was estimated by multiplying the mean weight of the fish in each section by the population estimate.

The short, slow-flowing section in Låktabäcken (maximum depth 1.4 m) was electrofished once during August 1983. During one night (August 23, 1983), three gill nets of multiple mesh sizes (Hammar and Filipsson 1985) were used in the longer pool section (maximum depth 3.1 m). In Bjuråsbäcken, six slow-flowing sections were electrofished once in August 1994 and in September 1994. In Storbäcken, nine (6–17 m) sections were surveyed.

Collected fish were anaesthetised, measured to the nearest millimetre (total length), and weighed to the nearest gram. Otholiths for age determination and data on sex and stage of sexual maturity were collected from 171 and 141 randomly sampled brown trout (older than yearling) in Låktabäcken and Bjuråsbäcken, respectively. Mean age and size at maturity were calculated according to Vøllestad et al. (1993). For Storbäcken brown trout, the catches were separated into age groups using the modes of the length–frequency histograms of the catches (this study) and length–age relationships determined from scales from a previous investigation (Södergren et al. 1973). The adult to juvenile growth ratio (Hutchings 1993) was calculated as the length at age 1+ in August (i.e., when no mature individuals occur) and adult growth rate was estimated as the length increment between age 1+ and age 3+.

To survey brown trout spawning locations in Låktabäcken, a 1.5-km-long section was electrofished in September of 1983 and 1984. The entire study area in Bjuråsbäcken was fished twice in September 1990. In addition, a 650-m-long section was fished three times in September 1994 (September 13, 22, and 30) to record ripe fish at the spawning grounds.

National Electrofishing Register

Description of included localities

Electrofishing data were collected during 1988–1994 from 5860 localities. The data were provided by regional authorities and fishery organisations in Sweden. The localities are distributed throughout Sweden and are generally 100–300 m^2 in size. Their altitude averaged 236 m above sea level, with extremes of 1 and 950 m. The stream order was generally low (1–4). The average stream width was 8 m (0.5–175 m) and the average depth 0.27 m (0.03–2.6 m).

The abundance of fish was determined using successive removal at 45% of the localities (Bohlin 1981). At localities where successive removal fishing had not been performed (55%), the total abundance of fish was estimated on the basis of average catch probabilities for the respective species and age groups. The classification of brown trout into age groups, i.e., yearlings (age 0+) versus older fish, was based on length frequencies. The fish were not sexed. At localities where brown trout was the only species caught, the population was designated as allopatric whereas brown trout populations occurring together with other species were designated as sympatric.

The physical data recorded at each locality were geographic location (latitude, longitude, and altitude), stream width, average depth, maximum depth, and air and water temperature. The bottom substrate was classified using five categories: (1) fine sand, (2) sand, (3) gravel, (4) stones, and (5) boulders. Water velocity was classified in the field using three categories: (1) slow sections (<0.2 m/s), (2) intermediate sections (0.2–0.7 m/s), and (3) fast sections (>0.7 m/s). Pool habitats were identified as localities with a low water velocity and riffle habitats as habitats with an intermediate or fast velocity. Further information was added during data entry including distance from each sampling locality to lakes upstream and downstream, the average air temperature (data from the Swedish Meteorological and Hydrological Institute) over the year, as well as in January and June, and catchment area (classified into five categories: <10, <100, <1000, <10 000, and ≥10 000 km^2).

Data analysis

To examine the occurrence of brown trout in relation to coexisting species, a data set consisting of 5860 localities was used. To be able to correctly compare abundance between the three creeks mentioned above and the larger National Electrofishing Register database, a subset of data from the same region, labelled northern Sweden, was selected north of 60°N at an altitude of 200–600 m. This subset consisted of 2427 localities and was also used for analysis of size distribution and growth. However, since the reports provided by the electrofishing crews sometimes lack information on specific parameters, the number of observations varies depending on the specific analysis. Statistical analysis was done with analysis of variance (ANOVA), analysis of covariance (ANCOVA), and linear regression.

Results

Three-creek study

Fish species composition and abundance

Brown trout occurred in both riffles and pools in Bjuråsbäcken (Table 2). Brown trout were also found in both habitats of Storbäcken, although they coexisted with bullhead and northern pike in pools and with bullhead in riffles. The size range of bullhead in riffles and pools was 20–85 and 90–125 mm, respectively, and of northern pike in pools was 75–295 mm. In Låktabäcken, brown trout dominated in the riffle habitat where a few grayling (size range 59–215 mm), burbot (116–240 mm), and minnows (50–90 mm) were caught, although densities of these other species never exceeded 1/100 m^2 (Table 2). However, no brown trout occurred in the slow-flowing parts of the creek. In the longer, slow-flowing section, grayling (11 individuals, size range 115–280 mm) and northern pike (five individuals, size range 142–347 mm) dominated the gillnet catches, which also provided three yellow perch and one burbot. Although not captured in the gill nets, minnows are regularly observed in this section. In the shorter of the pool sections of Låktabäcken, 21 grayling were caught in the electrofishing survey (size range 65–238 mm) together with one northern pike (length 150 mm).

Riffle density of older brown trout (>age 0+) was 15.1, 11.1, and 13.1/100 m^2 in Bjuråsbäcken, Storbäcken, and Låktabäcken, respectively. Brown trout biomass was 264 g/100 m^2 in Bjuråsbäcken and 279 g/100 m^2 in Storbäcken and Låktabäcken. No significant differences in riffle densities of older brown trout or in brown trout biomass were recorded between creeks (ANOVA, Tukey's HSD test, $p > 0.05$). Riffle density of age 0+ brown trout was significantly higher in Bjuråsbäcken than in the other creeks (44.8/100 m^2 versus 3.9

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Table 3. Age-specific lengths (mean and 95% confidence intervals) in August–September for brown trout in the study streams.

Age	Bjuråsbäcken		Storbäcken		Låktabäcken	
(years)	*n*	Length (mm)	*n*	Length (mm)	n	Length (mm)
0+	67	41.4±1.5	46	55.3±1.1	54	60.5±1.2
1+	79	86.6±1.8	83	110.3±1.8	256	111.1±0.7
2+	59	123.6±2.7	30	146.3±2.6	18	153.8±5.8
3+	30	161.1±6	12	176.4±3.7	16	189.1±7.9
4+	16	212.1±9	13	222.9±7	10	207.7±10
5+	6	261.3±11	8	281±23		

and 2.8/100 m^2 for Storbäcken and Låktabäcken, respectively).

Growth, size distribution, and sex ratio

Brown trout of age 0+ to 3+ were significantly smaller in each respective age-class (ANOVA, Tukey's HSD test, $p < 0.05$) in Bjuråsbäcken than in Låktabäcken and Storbäcken (Table 3). Age 5+ were larger in Storbäcken than in Bjuråsbäcken, although not significantly, while they were lacking in Låktabäcken. The annual proportional increase in length (sensu Hutchings 1993) declined from age 1+ to 3+ for brown trout in all creeks but remained on the same level from age 3+ to 5+ for Bjuråsbäcken and Storbäcken brown trout (Fig. 1). By contrast, it steadily declined with age in Låktabäcken brown trout. The adult to juvenile growth ratio was 0.7 for Låktabäcken and 0.86 for Bjuråsbäcken.

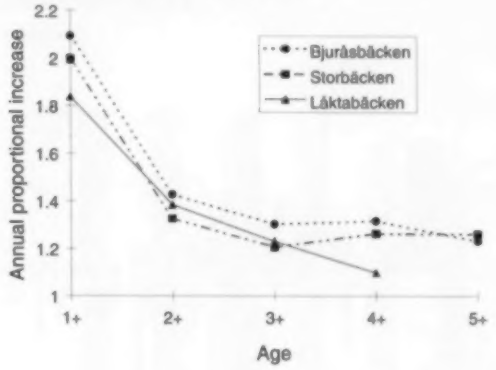
In the riffle habitat in Bjuråsbäcken, age-classes 0+, 1+, and 2+ were about equally represented, and 3% of the fish were longer than 170 mm (Fig. 2*a*). In the pool habitat in Bjuråsbäcken, age 0+ fish were lacking, while fish in the length interval 100–150 mm dominated. Twenty-four percent of the fish were longer than 170 mm. Most fish in riffle habitats in Låktabäcken and Storbäcken were in the size interval 100–125 mm (age 1+), with 7% of the fish being longer than 170 mm (Figs. 2*b* and 2*c*). In Låktabäcken, fish longer than 200 mm or older than age 4+ were very rare. In the pools in Storbäcken, fish less than 170 mm were lacking. Of the fish captured in this habitat, all were longer than 185 mm, and six of them were age 5+.

The male to female sex ratio was 1.00 in Låktabäcken. In Bjuråsbäcken the ratio was 1.19 in the riffles and 0.65 in the slow-flowing sections, a difference that was significant (χ^2-test, $p < 0.05$). Only one female (length 200 mm) of 32 (3%) inspected in the riffle habitats was sexually mature whereas 21 of 49 (43%) inspected in the pool habitats were mature.

Size and age at sexual maturation and spawning

Females matured earlier in Låktabäcken than in Bjuråsbäcken, but size at maturity did not differ (Table 4). Males also matured earlier in Låktabäcken but at a larger size. Females spawning in Bjuråsbäcken were significantly larger than those spawning in Låktabäcken (*t*-test, $p < 0.05$). The smallest mature females were 170 and 165 mm in Bjuråsbäcken and Låktabäcken, respectively. The size of mature males was similar in the two creeks, but older/larger ones were more common in Bjuråsbäcken than in Låktabäcken. The smallest mature males were 130 and 136 mm in Bjuråsbäcken and Låktabäcken, respectively.

Fig. 1. Annual proportional increase in length for brown trout in Bjuråsbäcken, Storbäcken, and Låktabäcken.



Electrofishing during spawning time along the entire study area in Bjuråsbäcken in 1990 only revealed ripe females at five locations. In September 1994, a 650-m-long reach of the study section, including two of the spawning areas from 1990, was electrofished on three occasions. On September 13, no ripe females were found. On September 22, however, two congregations of large females were recorded. Two of these females had previously been captured and fin-clipped in a slow-flowing section, indicating a migration from slow-flowing areas to riffle areas for spawning. Ripe males were encountered along the entire study section on all occasions. However, all large males (>170 mm) were recorded together with the congregations of females on September 22. By September 30, the large fish had left the spawning areas, indicating that spawning had been completed. In Låktabäcken, extensive electrofishing during the spawning periods in 1983 and 1984 also revealed congregations of ripe females. Ripe males could be found in all riffle areas.

National Electrofishing Register

Number of species and occurrence of brown trout

The number of species found at each electrofishing locality was positively correlated with both average and maximum depths of the stream and with its average width (linear regression for log-transformed cross-sectional area versus number of species, $r^2 = 0.09$, $p < 0.001$, $n = 2011$). A positive correlation was also found between catchment size and species number ($r^2 = 0.20$, $p < 0.001$, $n = 5372$). With increasing number of species, the proportion of localities with brown trout decreased (Fig. 3, ANOVA, $p < 0.001$, $n = 3956$). This effect was also significant in analyses where the effect of habitat size had been compensated for (ANCOVA, $p < 0.001$, with size of catchment area as covariate, $n = 3567$).

Abundance of brown trout

For localities in northern Sweden the yearling abundance was 9.4/100 m^2 in allopatric populations and 3.2/100 m^2 in sympatric ones (ANCOVA, $p < 0.001$, with stream width as covariate). The abundance of brown trout older than yearling was 12.6 and 3.7/100 m^2, respectively (ANCOVA, $p < 0.001$, with stream width as covariate). Thus, brown trout abundance was approximately three times higher in allopatric populations, a

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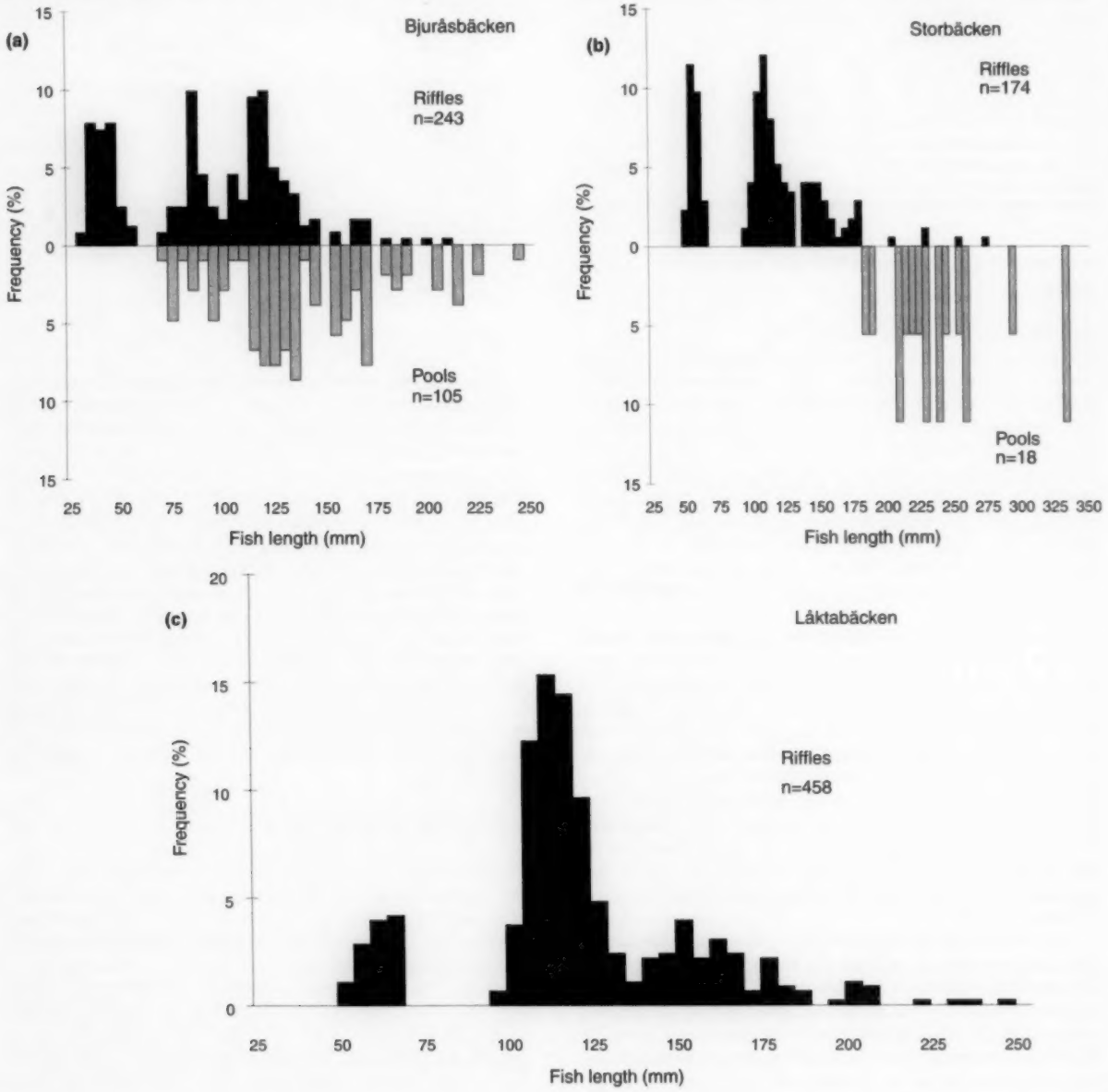
Fig. 2. Size distribution of brown trout in (*a*) pool and riffle habitats in Bjuråsbäcken, (*b*) pool and riffle habitats in Storbäcken, and (*c*) riffle habitats in Låktabäcken.



difference that was significant even when the effect of habitat size had been compensated for. In allopatric populations, older brown trout were significantly more abundant in pools than in riffles (ANCOVA, $p = 0.029$, with stream width as covariate), but no other significant differences in abundance were found between age groups or habitats.

Size and length distribution

A notable shift in size structure between riffle and pool habitats was detected in allopatric populations but not in sympatric ones (Fig. 4). The size of the largest brown trout individual at each electrofishing locality showed a significant positive correlation with the number of sympatric species. However, the variation explained by these factors was low; a significant multiple linear regression with only 13% explained variation consisted of seven variables, including average air temperature, maximum depth, and number of sympatric species, all with positive correlation ($p < 0.001$, $n = 1891$). Thus, number of sympatric species, climate, and habitat size all influenced the size of the largest brown trout individual.

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Table 4. Percentage of sexually mature brown trout, mean size (mm) of spawners, size range at maturity, and mean age (years) and size (mm) at first maturity in Bjuråsbäcken and Låktabäcken.

	Bjuråsbäcken				Låktabäcken			
	Females		Males		Females		Males	
Age (years)	*n*	%	*n*	%	*n*	%	*n*	%
1+	21	0	18	0	23	0	30	0
2+	29	0	27	24	29	7	31	71
3+	13	23	17	88	28	82	13	92
4+	6	100	10	100	11	100	6	100
Mean size of spawners	230		172		188		171	
Size range	170–400		130–320		165–217		136–227	
n	27		79		36		43	
Mean age at first maturation	3.18		2.28		2.57		2.01	
Mean size at first maturation	173.7		134.7		171.3		147.7	

Yearling growth

The only age group identified in the National Electrofishing Register is yearlings (age 0+). Yearlings grew faster when living in streams with several species. The size of yearling brown trout could be predicted with significant accuracy using number of sympatric species, distance to nearest upstream lake, day of the year, and average air temperature (multiple linear regression, $r^2 = 0.53$, $p < 0.001$, $n = 2281$). No correlation were found between yearling growth and the abundance of yearlings, the abundance of older brown trout, or stream size. Thus, climate, habitat factors, and biotic interactions were the main factors explaining brown trout population characteristics.

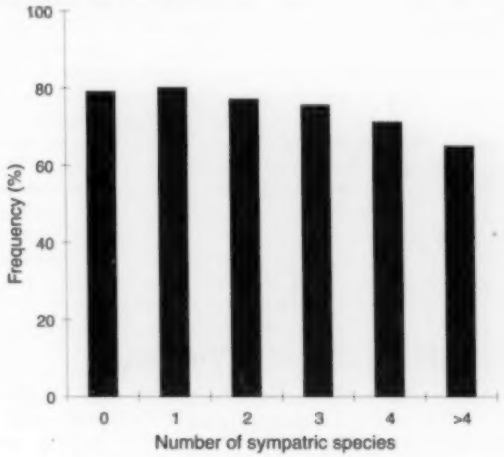
Discussion

It is evident that, on a larger geographic scale, the presence of other fish species affects the distribution of brown trout (this study; Degerman and Sers 1992). The frequency of occurrence and abundance of brown trout decrease if the number of coexisting species increases. However, brown trout populations may also be affected by biotic interactions on a smaller scale, i.e., within specific streams. We expected that habitat use and life history may be influenced. Although we have no direct evidence of predation or competition, the results of our study provided some indirect support for such a relationship.

Habitat use

The allopatric brown trout population in Bjuråsbäcken used all available habitats. There was a pronounced habitat shift, with nursery areas in riffle sections and older/larger brown trout favouring slow-flowing parts of the stream. This shift was verified with data from the National Electrofishing Register. The slow-flowing sections provide better growth opportunities for larger fish (cf. Jonsson 1985) and may also offer areas in which the risk of predation by terrestrial animals is reduced owing to increased cover (cf. Heggenes 1988). Since no yearling brown trout were recorded in the slow-flowing sections of Bjuråsbäcken, the habitat shift must take place after the first summer. Yearling brown trout have been shown to remain in the area of birth over the first summer (Gerking 1959; Näslund 1992).

Fig. 3. Frequency of localities that held brown trout associated with number of sympatric species. Data from the National Electrofishing Register; $n = 3956$ localities.



However, in the National Electrofishing Register, yearling abundance was lower in pools than in riffles, but the difference was not significant. This indicates that there may be no distinct boundaries between sympatric and allopatric populations on a larger scale and that, with respect to yearling habitat use, generalisations from single stream studies may be questionable.

In Bjuråsbäcken the habitat shift between riffles and pools was mainly undertaken by females. This is in line with the results of most studies of brown trout migration (e.g., Jonsson 1985). These studies all deal with brown trout migrating over longer distances, brown trout that migrate to the sea, a lake, or a larger stream. Females benefit more from such migration followed by enhanced growth than males do, since their fecundity is more directly dependent on body size (Jonsson 1985). However, these principles seem to be true also for the small-scale movements and habitat shifts within a small stream. Based on the size distribution of brown trout in the riffles, it may even be suggested that such a habitat shift is a prerequisite for the maturation of females in Bjuråsbäcken. This stream is small and shallow and it seems difficult for brown trout to grow larger than 170 mm (minimum size of mature females) in the riffle habitat, particularly if resources for reproduction have to be allocated. Thus the probability for female maturation in the riffle habitat of small streams seems to be low, which is supported by the fact that only one mature female was recorded in the riffle habitats prior to spawning in September. In addition, data from the National Electrofishing Register show that individuals above 170 mm constituted only 6% of the total number of allopatric brown trout in riffles.

The outmigration of females from riffle habitats is in line with the general statement that fish migrate when they are constrained by food limitations in their present habitat (Thorpe 1994). Brown trout are renowned for their strong intraspecific interactions, e.g., competition for feeding positions and cover, which can even be detected at early stages (Bachman 1984; Fausch 1984). Such interactions may be a major generating force behind the habitat shifts and brown trout distribution pattern in stream-resident, allopatric populations (Bohlin

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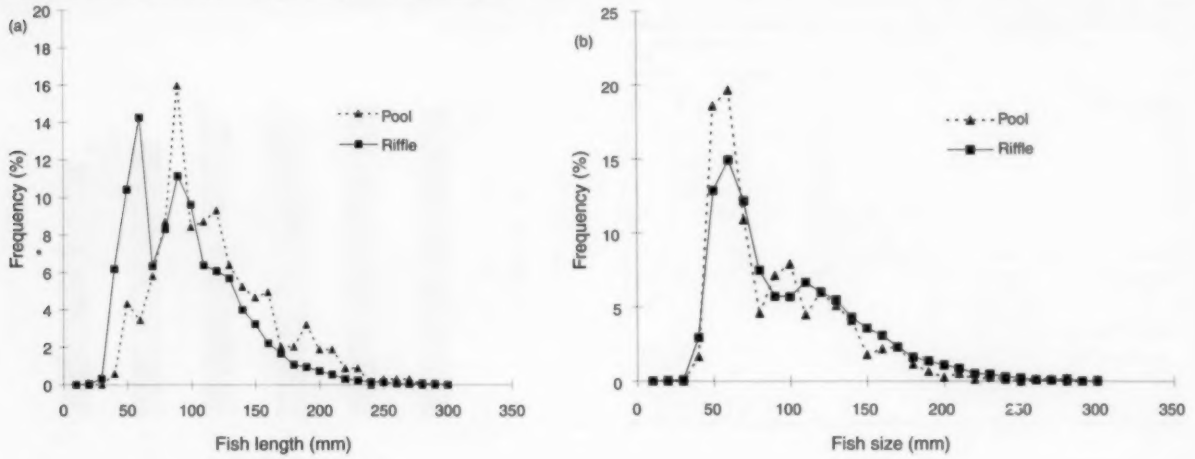
Fig. 4. Proportion of brown trout in different length-classes in pool and riffle habitats for (*a*) allopatric and (*b*) sympatric populations. Data from the National Electrofishing Register; $n = 1624$ localities.



1977). Although no differences in the abundance of older brown trout were found among the three creeks studied, brown trout densities in the National Electrofishing Register database were generally three times higher in allopatry than in sympatry, which lends some support to the above statement.

In Låktabäcken, with a multispecies fish community, the brown trout tended to remain in the riffle areas over its entire life cycle, probably as a result of the presence of other species in the slow-flowing habitats. This brown trout population seemed to be strictly resident, which also is indicated by the low rate of movement of older individuals both between and within years (Näslund 1992). Thus, in contrast with the situation in Bjuråsbäcken, females have to be able to attain maturity without leaving the riffle habitat for such a population to maintain itself. Låktabäcken had larger brown trout in the riffles than Bjuråsbäcken and also maturing females. This may be explained by the higher water depth of the riffles in Låktabäcken. Deeper water in the riffles may provide feeding habitat for somewhat larger fish (Heggenes 1988), and also for maturing females. Data from the National Electrofishing Register show that for sympatric brown trout populations, size distributions in riffles and pools are similar. Thus, the benefits of a habitat shift in terms of increased growth would seem to be limited.

However, in certain situations where the pressure from other species is less intense (Storbäcken), a size-dependent habitat shift may be possible for larger brown trout. This shift may be beneficial, since it may enhance the feeding conditions and availability of cover for larger fish (cf. Bachman 1984). One factor contributing to the benefits of the shift to deeper areas could be that large brown trout in Storbäcken are less vulnerable to predation from northern pike. The relatively small northern pike would not be able to prey on larger brown trout. Zalewski et al. (1985) found that predation pressure from European eel (*Anguilla anguilla*) and northern pike in streams was strongly reduced once brown trout reached a size of 170 mm, which was the minimum size of pool-dwelling brown trout in Storbäcken. The important role of size- and age-mediated habitat use by stream fish in relation to predation has been emphasised (Power 1987; Brown and Moyle 1991). It seems evident that riffles are efficient refugia from predation for small and young individuals (Brown and Moyle 1991) and for entire populations of stream fish species (this study; Degerman and Sers 1993; Fraser et al. 1995). The impact of coexisting northern pike on habitat use by brown trout has been shown in laboratory experiments and in the field (Degerman and Sers 1993; Greenberg 1994). Brown trout will reduce their use of slow-flowing sections if northern pike are present. The presence of burbot seems to have similar effects on yearling brown trout (Degerman and Sers 1993). Thus, predation risk seems to be an important factor affecting habitat use, and hence, growth and survival, in stream-dwelling brown trout.

On the other hand, competition in streams with the kind of species composition found in Låktabäcken is less well understood (Degerman and Sers 1993). A generally low competitive ability in the brown trout is reflected in its low occurrence (this study) or abundance in multispecies communities (Degerman and Sers 1992). Although grayling and brown trout can use the same microhabitat, little is known about their specific competitive interactions (Greenberg et al. 1996). The effects of competition from other species may add to the inhospitableness of predator-rich, slow-flowing stretches, thereby discouraging brown trout from using such habitats. However, these competitive effects remain to be quantified.

Life history

Growth differences are a central key to understanding interpopulational variation in life history. In our study the early growth of brown trout (ages 0–3+) was more rapid in sympatry than in allopatry, but since growth is strongly correlated with temperature, food availability, etc., it might be erroneous to generalise. In addition, growth can be affected by abundance (Newman 1993). Thus, higher densities in small streams could explain the slower growth of brown trout in these streams. Support for the general conclusion that juvenile growth is more rapid in sympatry can be found in data from the National Electrofishing Register. They show that the growth of yearlings, and possibly other age-classes, increases with the number of

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coexisting fish species. In addition, no correlation between growth and brown trout abundance was found.

For adult brown trout (>age 3+) the positive effects of the habitat shifts on growth in Bjuråsbäcken and Storbäcken were evident. In contrast with Låktabäcken brown trout, the annual proportional increase in length remain unchanged after 3 years of age. However, in the data from the National Electrofishing Register database the largest brown trout found increased in size with the number of sympatric species, i.e., in spite of increasing biotic interactions. The explanation is probably that these environments are more productive, thus allowing the few brown trout that survive to grow fast and reach a large size. Still, the average life span of stream-resident brown trout in sympatric populations is probably shorter than that of allopatric populations. Although no estimates of survival were obtained in the streams studied here, it is clear from size distributions that the proportion of bigger, and presumably older, fish is larger where a shift in habitats can occur.

Calculating the adult to juvenile growth ratio in accordance with Hutchings (1993) reveals a relatively small difference among creeks, but the lowest ratio was recorded in Låktabäcken where the fish mature early. This is in line with the results of Hutchings (1993), who concluded that age at maturity was positively associated with the adult to juvenile growth rate at the population level. Furthermore, it is evident that brown trout in Bjuråsbäcken, which seem to have a higher adult survival, also mature later. In addition, the female spawners are larger in Bjuråsbäcken. An environment associated with high adult mortality, such as Låktabäcken, will select for increased reproductive effort and decreased age at maturity whereas an environment associated with high juvenile mortality rates, such as Bjuråsbäcken, will select for increased age at maturity, decreased reproductive effort, and longer life span (Schaffer 1974; Hutchings 1993). Schaffer (1974) predicted that environments characterised by increased growth during potentially reproductive ages, as is the case in Bjuråsbäcken, will select for increased age at maturity. All of these observed differences in life history traits are in accordance with predictions based on life history theory (cf. Reznick et al. 1990).

In this study, we have used detailed data from studies of three specific populations and combined them with results from a large database. Even if this approach has advantages, it also has some limitations when it comes to generalisations. It gives no direct evidence of predatory or competitive mechanisms, and several factors, apart from predation and competition, may affect habitat use and life history of stream-dwelling fish. Differences in physical factors such as water quality, temperature, etc., may also have influenced some of the results (e.g., growth) of the three-creek study. Moreover, the National Electrofishing Register database is too blunt an instrument to discriminate small differences in habitat preferences and it contains no data on fish age or sexual maturity. However, the large data set provides a very high number of observations, and thus stable results, and we believe that the three intensively studied creeks are comparable in terms of physical factors and representative of the type of streams discussed. Thus, the results provide some support for the hypothesis that habitat use and life history of brown trout are influenced by fish species interactions. Allopatric brown trout seemed to utilise more habitats, have a higher adult survival rate, grow more slowly as juveniles, and mature later than sympatric brown trout.

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An experimental analysis of crayfish (*Orconectes rusticus*) effects on a *Dreissena*-dominated benthic macroinvertebrate community in western Lake Erie

Timothy W. Stewart, Jeffrey G. Miner, and Rex L. Lowe

Abstract: Quantitative descriptions of trophic interactions between *Dreissena* (zebra and quagga mussels) and other organisms are needed for an understanding of *Dreissena*'s effects on energy flow and community dynamics in the Great Lakes. We used a field experiment to quantify effects of crayfish (*Orconectes rusticus*) predation on a *Dreissena*-dominated benthic macroinvertebrate community in western Lake Erie. Rocks colonized by *Dreissena* and associated macroinvertebrates were placed in cages and cageless reference plots located at a depth of 4 m. Crayfish (0, 8.3, and 20.8 individuals·m^{-2}) placed in cages were allowed to graze for 28 days. *Dreissena* had a greater effect than crayfish on the macroinvertebrate community, with positive relationships observed between *Dreissena* densities and both total macroinvertebrate biomass and densities of the amphipod *Gammarus fasciatus*. However, crayfish at densities of 20.8 individuals·m^{-2} still reduced non-*Dreissena* macroinvertebrate biomass and *Gammarus* densities by 33 and 37%, respectively, relative to crayfish exclosures. Crayfish had negligible effects on *Dreissena* densities or shell length frequency distribution. Because crayfish and amphipods are prey for several fish species, trophic interactions among *Dreissena*, amphipods, and crayfish may be important in channeling energy from *Dreissena* to higher trophic levels in the Great Lakes.

Résumé : Des descriptions quantitatives des interactions trophiques entre *Dreissena* (moule zébrée et moule quagga) et d'autres organismes sont nécessaires pour comprendre les effets de *Dreissena* sur le flux d'énergie et la dynamique des communautés dans les Grands Lacs. Nous avons réalisé une expérience sur le terrain pour quantifier les effets de la prédation par une écrevisse (*Orconectes rusticus*) dans une communauté benthique de macro-invertébrés dominée par *Dreissena* dans l'ouest du lac Érié. Des roches colonisées par *Dreissena* et des macro-invertébrés associés ont été placées dans des cages et dans des parcelles témoins sans cage à une profondeur de 4 mètres. Les écrevisses (0, 8,3, 20,8 individus · m^{-2}) placées dans les cages ont pu s'alimenter durant 28 jours. *Dreissena* a eu un effet plus marqué que les écrevisses sur la communauté de macro-invertébrés; on a observé une relation positive entre les densités de *Dreissena*, d'une part, et la biomasse totale des macro-invertébrés et les densités de l'amphipode *Gammarus fasciatus*, d'autre part. Toutefois, à des densités de 20,8 individus · m^{-2}, les écrevisses ont réduit la biomasse des macro-invertébrés autres que *Dreissena* ainsi que la biomasse de *Gammarus* de 33 % et de 37 %, respectivement, par rapport à la situation où il y avait absence d'écrevisses. Les écrevisses ont eu des effets négligeables sur les densités de *Dreissena* ou la distribution statistique des longueurs des coquilles. Étant donné que les écrevisses et les amphipodes sont des proies pour plusieurs espèces de poisson, les interactions trophiques entre *Dreissena*, les amphipodes et les écrevisses peuvent être importantes dans la canalisation du flux d'énergie à partir de *Dreissena* jusqu'aux niveaux trophiques supérieurs dans les Grands Lacs.
[Traduit par la Rédaction]

Introduction

Interactions among predators, prey, and organisms that influence the nature and strength of predator–prey interactions are important structuring agents of biological communities (Polis and Strong 1996). In marine rocky intertidal communities, predators that forage efficiently or occur at high densities can reduce the abundance of prey organisms (Schmitt 1987). However, benthic resource enhancement by sessile macroinvertebrates can reduce predator effects on benthic prey abundance (Gosselin and Chia 1995). For example, structurally complex habitat created by bivalve shells can cause increased abundance of small organisms by providing refugia from predation and other disturbances (Gosselin and Chia 1995; Blanchette 1996). Additionally, organic matter accumulating in spaces between bivalve shells may provide both food and cover (Gosselin and Chia 1995; Blanchette 1996). Thus, the mechanisms structuring energy flow and species abundance in benthic communities can be better understood by quantifying the relative effects of predators and resource enhancement by sessile macroinvertebrates (Gosselin and Chia 1995).

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In the late 1980s, the Laurentian Great Lakes were invaded by two species of sessile bivalves, the zebra mussel (*Dreissena polymorpha*) and the quagga mussel (*Dreissena bugensis*). *Dreissena* is the first abundant sessile macroinvertebrate to inhabit rocky substrates in the Great Lakes, with population densities of 2000 – 50 000 individuals·m^{-2} recorded in nearshore habitats (Dermott et al. 1993; Griffiths 1993; Stewart and Haynes 1994; Wisenden and Bailey 1995). Recent declines in phytoplankton biomass with concomitant increases in benthic algal biomass suggest that *Dreissena* redirects energy and nutrients from pelagic to benthic communities (Nicholls and Hopkins 1993; Lowe and Pillsbury 1995). This apparent shift in the relative abundance of benthic and pelagic components of Great Lakes food webs has caused concern for the welfare of commercially important fish species (Mitchell et al. 1996). Quantitative descriptions of trophic interactions within Great Lakes food webs are needed to develop a predictive understanding of *Dreissena*'s ultimate impacts on commercially important species of fish.

With a few exceptions (Wisenden and Bailey 1995; Nalepa et al. 1996), *Dreissena* has caused increased biomass and densities of benthic macroinvertebrates in the Great Lakes (Dermott et al. 1993; Griffiths 1993; Stewart and Haynes 1994; Botts et al. 1996). *Dreissena* may have positive effects on other macroinvertebrates because shells increase surface area of hard substrate and create structurally complex substrates that provide smaller macroinvertebrates with refugia from predation and other disturbances (Botts et al. 1996; T.W. Stewart, unpublished data). Additionally, filter-feeding *Dreissena* may increase benthic organic matter by depositing pseudofeces and feces and by stimulating benthic algal production through excreted nutrients and increased benthic light levels (Lowe and Pillsbury 1995; T.W. Stewart, unpublished data). These sources of benthic organic matter are valuable food and habitat for other benthic macroinvertebrates (Izvekova and Lvova-Katchanova 1972; Lamberti 1996)

There is evidence that crayfish densities also have increased in the Great Lakes following the *Dreissena* invasion (Stewart and Haynes 1994). Two potential mechanisms for recent increases in crayfish densities include (*i*) crayfish predation on *Dreissena* (representing a direct positive effect of *Dreissena* on crayfish) and (*ii*) crayfish predation on other benthic macroinvertebrates that have increased in abundance because of *Dreissena*-generated resource enhancement (an indirect positive effect of *Dreissena* on crayfish; Momot 1995; Perry et al. 1997). Because crayfish and other macroinvertebrates are important prey for several species of fish that occur in the Great Lakes (Lodge and Hill 1994; Lott et al. 1996), direct and indirect interactions between crayfish and *Dreissena* may be important in channeling energy from *Dreissena* to benthivorous fish.

The primary objective of this study was to quantify effects of crayfish (*Orconectes rusticus*) on a *Dreissena*-dominated benthic macroinvertebrate community in western Lake Erie. Quantitative estimates of interaction strength among crayfish, *Dreissena*, and other macroinvertebrates that we obtained are used to explain community properties and energy flow dynamics in the Great Lakes under post-*Dreissena* invasion conditions. To our knowledge, this is the first quantitative investigation of predator effects on benthic communities in the Great Lakes.

Materials and methods

Study area

The study site was in western Lake Erie, near Put-in-Bay, Ohio. Benthic habitat at the study site was typical of nearshore zones in western Lake Erie, dominated by limestone/dolomite cobble among patches of smaller particles. The experiment was conducted using SCUBA at a depth of 4.0 m in an area where surfaces of cobble not buried in sediments were covered with *Dreissena* at least one layer thick.

Experimental design

A completely randomized design was used with four treatments ($n = 5$ replicates each). Fifteen cages enclosing horizontal benthic surface areas of 0.24 m^2 were placed at the study site. Crayfish (adult male *O. rusticus*, 26–32 mm carapace length) were placed in cages at zero, low, or high densities. Two individuals were placed in cages of the low crayfish density treatment (8.3 crayfish·m^2 horizontal benthic surface area^{-1}) and five individuals were placed in cages of the high crayfish density treatment (20.8 crayfish·m^{-2}) on July 5, 1995. No crayfish were placed in cages constituting the crayfish exclosure treatment. Crayfish densities used in this experiment were within the range of densities recorded in the Great Lakes following the *Dreissena* invasion (Stewart and Haynes 1994) and were similar to crayfish densities estimated from an August 1995 survey of our study site (mean ± SE = 14.6 ± 2.5 individuals·m^{-2}, $n = 10$). This survey was conducted by placing a 0.24-m^2 frame on the lake bed and counting all crayfish observed while turning over rocks located within the frame. Crayfish density estimates obtained in our survey were conservative because turbidity caused by disturbance of underlying sediments made it difficult to see crayfish when overturning rocks. Thus, actual densities at the study site may have been equal to or exceeded densities used in the high crayfish density treatment. *Orconectes rusticus* was the only crayfish species collected at our study site.

Cages were constructed of iron frames that were covered on bottoms and sides with nylon window screening (1.7-mm mesh) and with clear acrylic lids. Cage bottoms were covered with an 8-L mixture of silt, sand, and gravel collected from the study site. A volume of *Dreissena*-covered rocks sufficient to fill an 8-L bucket was then collected from the study site and placed in each cage. The same volume of *Dreissena*-covered rocks was also placed in five 0.24-m^2 cageless reference plots after rocks originally contained within these plots were removed. Rocks were left within cages and cageless plots for 10 days before the experiment began to allow communities upon rocks to recover from disturbances incurred during the experiment setup. One rock was removed from each cage and cageless plot on the day the experiment began for purposes of quantifying preexperimental densities of *Dreissena* and other macroinvertebrates. We estimated the total surface area of rocks in cages and cageless plots (mean ± SE = 0.24 ± 0.01 m^2) by collecting four bucketfuls of rocks (minus one rock to account for rocks removed from cages and cageless plots during preexperimental sampling), removing *Dreissena* from rocks, wrapping each rock in aluminum foil, weighing the foil, and then converting foil weights to area measurements using an equation based on a foil weight-to-area curve. Initial *Dreissena* densities in cages and cageless plots (mean ± SE = 6203 ± 456 individuals·m^2 horizontal benthic surface area^{-1}) were estimated by multiplying the number of *Dreissena* per rock surface area in preexperimental samples by the mean value for total rock surface area in cages and cageless plots.

Macroinvertebrate samples were collected before the experiment began and at the end of the 28-day experiment by transferring one rock from each cage or cageless plot to a polyethylene bag and transporting the sample to the water surface. Ten crayfish were also recaptured from enclosures at the end of the experiment to examine stomach contents and verify the occurrence of macroinvertebrates in crayfish diets. Macroinvertebrate samples and crayfish were fixed and preserved in 10% buffered formalin. Upon returning to the laboratory, formalin in samples was replaced with 70% ethanol.

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Processing of macroinvertebrate samples

Macroinvertebrate samples were processed by removing *Dreissena* and other macroinvertebrates from rocks and then sieving sample contents through a 500-μm mesh. Sieve contents were scanned under 10× power and counts of individuals within each taxon were made. Densities of *Dreissena* and other macroinvertebrates (number of individuals per square metre of rock surface area) were determined after conducting macroinvertebrate counts and estimating rock surface area. Total macroinvertebrate biomass minus *Dreissena* (hereafter, "total macroinvertebrate biomass," grams ash-free dry mass (AFDM) per square metre of rock surface area) was determined by drying macroinvertebrates at 60°C for 24 h and then ashing at 550°C for 24 h (APHA 1989). *Dreissena* shell lengths were measured to the nearest 1.0 mm. Finally, we examined crayfish stomach contents after removing, opening, and irrigating stomachs with water to flush out stomach contents.

Monitoring cage effects

To minimize cage effects on the macroinvertebrate community, we visited the site weekly and removed fouling material from cage exteriors. We documented potential cage effects by measuring light levels and periphyton AFDM within cages and cageless reference plots. Light levels were measured in cages and cageless plots at the conclusion of the experiment. Clay flowerpots providing substrates for sampling periphyton were placed in cages and cageless plots at the same time that *Dreissena*-covered rocks were placed in cages and cageless plots. Clay pots were collected before the experiment began and at the end of the experiment by transferring pots to polyethylene bags and transporting them to the surface. These periphyton samples were maintained in the dark within coolers filled with ice and were frozen upon return to the laboratory. After each periphyton sample was thawed, macroinvertebrates visible to the unaided eye were removed from the sample. Periphyton AFDM was then determined using standard methods (APHA 1989).

Data analysis

We analyzed data that were collected before the experiment began to document preexperimental conditions within cages and cageless reference plots. We found that *Dreissena* density was an important determinant of total macroinvertebrate biomass (linear regression; adjusted $R^2 = 0.60$, $P = 0.001$) and densities of *Gammarus fasciatus* (adjusted $R^2 = 0.72$, $P < 0.001$). *Gammarus* was the dominant non-*Dreissena* macroinvertebrate in terms of numbers (92%) and biomass (87%) at the beginning of the experiment. Therefore, we controlled for effects of *Dreissena* by including *Dreissena* density as a covariate in analysis of covariance (ANCOVA) and Tukey's multiple comparisons tests for treatment effects on total macroinvertebrate biomass and macroinvertebrate densities. However, before using *Dreissena* density in ANCOVAs, we made sure that mean *Dreissena* shell length did not differ among treatments because *Dreissena* shell surface area is highly correlated with shell length, and the amount of habitat provided to macroinvertebrates per individual *Dreissena* shell would differ if mean shell length differed among treatments. No among-treatment differences in *Dreissena* densities, mean shell length (overall mean shell length ± SE = 9.8 ± 0.42 mm, range = 1–22 mm), or periphyton AFDM existed before the experiment began (analyses of variance (ANOVAs); $P = 0.922$, $P = 0.294$, and $P = 0.359$, respectively). Additionally, total macroinvertebrate biomass and macroinvertebrate densities did not differ among treatments when the experiment began (ANCOVAs; $P = 0.553$ and $P \geq 0.351$, respectively).

We used ANCOVA to detect differences among treatments even when *Dreissena* densities varied. However, because crayfish density is actually a continuous variable, we also were able to use multiple linear regression to quantify the relative strength of effects of crayfish and *Dreissena* on macroinvertebrate biomass and densities while statistically controlling for cage effects. In regression analyses, we used the mean crayfish density estimate from the August 1995 survey as the crayfish density in the cageless reference treatment. To quantify cage effects, which is a categorical variable (Neter et al. 1983), values of 0 and 1 were used in regressions to indicate the absence or presence, respectively, of a cage. Standardized regression coefficients were compared to show the relative strengths of effects of multiple independent variables on the same dependent variable (Mitchell 1993). Variance inflation factors, describing the degree of collinearity (i.e., correlations among independent variables), never exceeded 1.2 in any of our regression outputs, suggesting that collinearity problems were not severe in our regression analyses (McClendon 1994).

Prior to conducting statistical analyses, all data were log-transformed to meet normality assumptions. Data from two replicates of the low crayfish density treatment were excluded from statistical analyses due to damage occurring to cages during the experiment. All statistical analyses were conducted using SYSTAT 5.2.1 (Wilkinson 1992), and effects were considered statistically significant at $P \leq 0.05$. To maintain the probability of committing type I errors at $P \leq 0.05$ when conducting several univariate tests on the same data set simultaneously, we used Bonferroni corrections ($P = 0.05$/number of dependent variables) to adjust the critical value for rejecting null hypotheses (Scheiner 1993).

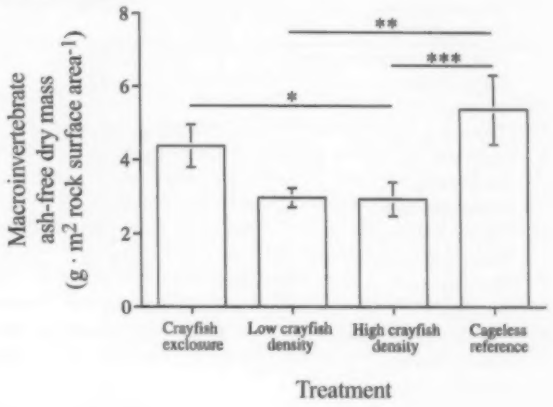

Fig. 1. Total macroinvertebrate biomass minus *Dreissena* (mean ± SE) in the four treatments. Lines are drawn above bars where statistically significant differences existed between treatments: significantly different at the *95, **99, and ***99.9% confidence levels, respectively. Macroinvertebrate biomass in crayfish exclosure and low crayfish density treatments did not differ ($P = 0.070$) due to the small number of replicates ($n = 3$) of the latter treatment.

Results

Treatment effects on total benthic macroinvertebrate biomass

Total macroinvertebrate biomass (minus *Dreissena*) differed among treatments at the end of the experiment (ANCOVA; $F = 11.4$, df = 3, 13, $P = 0.006$). As expected, total macroinvertebrate biomass also changed as a function of the covariate, *Dreissena* density ($F = 79.3$, df = 1, $P < 0.001$), reflecting the importance of *Dreissena*. Total macroinvertebrate biomass was 30 and 33% lower in the low and high crayfish density treatments, respectively, relative to the crayfish exclosure treatment (Tukey's test; $P = 0.070$ and 0.011) (Fig. 1). Although not statistically significant, results from the three

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Table 1. Macroinvertebrate densities (mean number of individuals·m^2 rock surface area^{-1}, with SE in parentheses) in each treatment.

Taxon	Treatment			
	Crayfish exclosure	Low crayfish density (L)	High crayfish density (H)	Cageless reference
Cnidaria				
Hydridae	555 (154)	375 (134)	417 (186)	1 155 (231)
Platyhelminthes				
Dugesia tigrina	715 (279)	457 (176)	395 (133)	1 873 (377) H
Annelida				
Alboglossiphonia heteroclita[a]	95 (95)	0	0	0
Oligochaeta	71 (55)	0	10 (10)	198 (101)
Mollusca				
Dreissena spp.[b]	10 500 (1430)	9 361 (1829)	9 592 (1566)	11 922 (2455)
Ferrissia sp.	820 (290)	428 (172)	1 110 (153)	2 046 (380) L
Physella sp.	47 (21)	28 (28)	0	123 (100)
Gyraulus parvus[a]	44 (23)	14 (14)	0	20 (20)
Amnicola limosa	43 (37)	0	40 (32)	34 (34)
Elimia livescens	22 (14)	0	0	20 (20)
Arthropoda				
Acarina	0	7 (7)	0	90 (90)
*Gammarus fasciatus****	32 641 (4409) H	23 241 (2089)	20 484 (3506)	33 189 (7865)
Caecidotea sp.	0	0	0	20 (20)
Psephenus sp.	0	17 (17)	0	0
Chironomidae	193 (100)	40 (30)	73 (30)	1296 (253)
Heptageniidae	18 (13)	40 (30)	15 (15)	0
Polycentropus sp.	0	0	30 (19)	11 (11)

Note: ***Statistically significant relationship between *Dreissena* and macroinvertebrate densities at the 99.9% confidence level after Bonferroni adjustment of the critical *P*-value ($P = 0.05/16$). Macroinvertebrate densities were greater ($P \leq 0.05$) in the designated treatment than in the treatment indicated by H and L if Bonferroni correction of the critical *P*-value was not considered.

[a]Homogeneity of slopes assumption (Wilkinson 1992) was not met for these taxa. Thus, ANCOVAs were not conducted.

[b]*Dreissena* spp. density was used as the covariate in ANCOVAs.

replicates at low crayfish densities closely reflect the effects of high crayfish densities on total macroinvertebrate biomass. Total macroinvertebrate biomass was also greater in the cageless reference treatment than in the low and high crayfish density treatments ($P = 0.007$ and $P = 0.001$, respectively) (Fig. 1). Although these results identify a cage effect on macroinvertebrate biomass, we were still able to evaluate the effects of crayfish on macroinvertebrate biomass by comparing responses among cage treatments.

Treatment effects on macroinvertebrate densities

A statistically significant positive relationship existed between *Dreissena* densities and densities of the amphipod *G. fasciatus* (ANCOVA; $P < 0.001$) (Table 1). *Gammarus* constituted 83% of the biomass and 90% of the numbers of non-*Dreissena* macroinvertebrates at the end of the experiment (Table 1). Because we analyzed for treatment effects on 16 macroinvertebrate taxa, the critical value for rejecting the null hypothesis of no treatment effect on macroinvertebrate densities was very low after Bonferroni adjustment of the critical *P*-value ($P \leq 0.003$) (Table 1). If the Bonferroni critical *P*-value adjustment was not made, statistically significant treatment effects on densities of the flatworm *Dugesia tigrina*, the limpet *Ferrissia* sp., and *Gammarus* were observed (ANCOVAs; $P = 0.046$, $P = 0.028$, and $P = 0.016$, respectively) (Table 1). These three taxa comprised 96% of the numbers and biomass of all non-*Dreissena* macroinvertebrates collected at the end of the experiment. Thus, treatment effects on these taxa contributed substantially to treatment effects on total macroinvertebrate biomass (Fig. 1). *Gammarus* densities were 37% lower in the high crayfish density treatment than in the crayfish exclosure treatment, suggesting that high densities of crayfish reduced *Gammarus* densities (Tukey's test; $P = 0.010$) (Table 1). Only *Dugesia* and *Ferrissia* densities were significantly greater in the cageless reference treatment than in any cage treatment, although densities of these taxa did not differ among cage treatments (Table 1). This suggests that cages had negative effects on *Dugesia* and *Ferrissia* but that interactions between these taxa, *Dreissena*, and crayfish were weak.

Treatment effects on *Dreissena*

Dreissena densities and mean *Dreissena* shell length (overall mean shell length ± SE = 11.03 ± 0.20 mm, range = 1–24 mm) did not differ among treatments at the end of the experiment (ANOVAs; $F = 0.27$, df = 3, 14, $P = 0.844$ and $F = 0.66$, df = 3, 14, $P = 0.588$, respectively) (Table 1). Among-treatment differences in the *Dreissena* shell length frequency distribution did occur at the end of the experiment ($\chi^2 = 61.8$, df = 42, $P < 0.05$). Results from subdividing the chi-square analysis (Zar 1984) indicated that the shell length frequency distribution in the low crayfish density treatment was different from the shell length frequency distribution in the other three treatments. Only 45% of *Dreissena* in the low crayfish density treatment had shell lengths ≤12 mm whereas 63% of *Dreissena*

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in crayfish exclosures had shell lengths ≤12 mm. These differences follow expectations based on observations that crayfish preferentially prey upon small *Dreissena* (Perry et al. 1997). However, the shell length frequency distribution in the high crayfish density treatment did not differ from the shell length frequency distribution in crayfish exclosure and cageless reference treatments ($\chi^2 = 36.1$, df = 28, $P > 0.10$). Because the *Dreissena* shell length frequency distribution also differed among treatments at the beginning of the experiment (χ^2; df = 48, $P < 0.001$), preexperimental variation in the *Dreissena* shell length frequency distribution among rocks may have overwhelmed crayfish effects on the shell length frequency distribution. Thus, although crayfish may have preyed on *Dreissena*, we conclude that crayfish had negligible effects on *Dreissena*.

Effects of crayfish, *Dreissena*, and cages on other macroinvertebrates

Dreissena, crayfish, and cages all had statistically significant effects on total macroinvertebrate biomass, together explaining 90% of the variation in total macroinvertebrate biomass (Table 2). Standardized regression coefficients suggest that *Dreissena* had the greatest effect on total macroinvertebrate biomass (standardized coefficients from regressing total macroinvertebrate biomass on *Dreissena*, crayfish, and cage = +0.73, –0.34, and –0.48, respectively) (Table 2). Both crayfish and cages had strong negative effects on total macroinvertebrate biomass (Table 2).

Both crayfish and *Dreissena* were important determinants of *Gammarus* densities, together explaining 84% of the variance in *Gammarus* densities (Table 3). The variable "cage" was dropped from the *Gammarus* regression model because cages did not have statistically significant effects on *Gammarus* densities (multiple linear regression; $P = 0.066$). Additionally, inclusion of this variable in the regression model would have only increased the explanatory power (R^2) of the model by 3%. Positive effects of *Dreissena* were most important in determining *Gammarus* densities (standardized coefficient = +0.87), but negative effects of crayfish were also important (standardized coefficient = –0.29) (Table 3). Because *Gammarus* constituted 83% of the total biomass of non-*Dreissena* macroinvertebrates at the end of the experiment, statistically significant effects of *Dreissena* and crayfish on total macroinvertebrate biomass were largely attributed to effects of these taxa on *Gammarus*. In contrast with the regression results with *Gammarus*, only cages had statistically significant effects on densities of *Dugesia* and *Ferrissia*, but cage effects explained only 37 and 25% of the variation in densities of these taxa, respectively (Table 3).

Mechanisms for crayfish and cage effects on macroinvertebrates

Gammarus constituted 94% of all macroinvertebrates identified in crayfish stomachs, while water mites (Acarina), chironomids, and heptageniid mayflies were also found in crayfish stomachs. Occurrence of *Gammarus* in crayfish diets provides supporting evidence that predation is an important mechanism for crayfish-generated reductions in *Gammarus* densities and total macroinvertebrate biomass.

Light levels were lower in cages than in cageless reference plots at the conclusion of the experiment (separate variances

Table 2. Total macroinvertebrate biomass as a function of *Dreissena*, crayfish, and cages (model adjusted $R^2 = 0.90$).

Independent variable	Regression coefficient	SE	Standardized regression coefficient	*P*-value
Intercept	–1.63	0.28		
Dreissena density	+0.62	0.07	+0.73	<0.001
Crayfish density	–0.08	0.02	–0.34	0.001
Cage	–0.14	0.03	–0.48	<0.001

$T = -13.27$, df = 2.7, $P = 0.002$). Light attenuation by cage walls and lids provides a likely explanation for among-treatment differences in periphyton AFDM observed at the conclusion of the experiment (ANOVA; $F = 33.41$, df = 3, 13, $P < 0.001$). Indeed, periphyton AFDM was greater in the cageless reference treatment than in the caged treatments (Tukey's test; $P = 0.002$ in all multiple comparisons) but did not differ among cage treatments ($P \geq 0.90$). These results suggest that periphyton production was reduced within cages relative to cageless reference plots, providing one potential explanation for cage effects on *Dugesia* and *Ferrissia* (Table 3).

Discussion

Although resource enhancement by *Dreissena* has strong positive effects on benthic macroinvertebrate biomass and densities of *Gammarus* in western Lake Erie, crayfish also have strong negative effects on non-*Dreissena* macroinvertebrate biomass and *Gammarus* densities. Because crayfish and amphipods are prey for several species of fish that inhabit the Great Lakes (Lodge and Hill 1994; Lott et al. 1996), trophic interactions among *Dreissena*, amphipods, and crayfish may be important in channeling energy from *Dreissena* to benthivorous fish.

Strong positive effects of *Dreissena* on amphipod densities and macroinvertebrate biomass have been observed in other studies, and amphipods are typically the dominant component of macroinvertebrate communities on rocky substrates in the Great Lakes that are inhabited by *Dreissena* (Dermott et al. 1993; Griffiths 1993; Stewart and Haynes 1994). Structurally complex microhabitat provided by shells is an important mechanism for *Dreissena*-generated increases in biomass and densities of amphipods (Botts et al. 1996; T.W. Stewart, unpublished data). Amphipods may derive protection from predators, strong currents, and wave stress by inhabiting interstitial spaces between *Dreissena* shells and other coarse substrate particles (Botts et al. 1996; Dahl and Greenberg 1996).

Despite refugia potentially provided by *Dreissena* shells, predation by crayfish can reduce total macroinvertebrate biomass and amphipod densities in western Lake Erie. The general importance of amphipods and other macroinvertebrates in crayfish diets is a source of disagreement (Momot 1995). Lodge et al. (1994) and Nystrom et al. (1996) concluded that amphipods can easily elude crayfish and and are therefore typically rarer in crayfish diets than gastropods and other less mobile taxa. However, crayfish have preyed on amphipods and reduced their densities in some systems lacking *Dreissena* (Hanson and Chambers 1995; Momot 1995). Additionally, Momot (1995) concluded that crayfish prefer animal protein

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Table 3. Macroinvertebrate densities as a function of *Dreissena*, crayfish, and cages.

Dependent variable (taxon)	Independent variable	Regression coefficient	SE	Standardized regression coefficient	*P*-value	Model adjusted R^2
Gammarus fasciatus	Intercept	+0.42	0.46			
Gammarus fasciatus	*Dreissena* density	+1.02	0.11	+0.87	<0.001	
Gammarus fasciatus	Crayfish density	–0.10	0.03	–0.29	0.010	0.84
Dugesia tigrina	Intercept	+3.22	0.17			
Dugesia tigrina	Cage	–0.65	0.20	–0.63	0.005	0.37
Ferrissia sp.	Intercept	+3.26	0.14			
Ferrissia sp.	Cage	–0.43	0.17	–0.54	0.021	0.25

Note: Only independent variables having statistically significant effects ($P \leq 0.05$) on the dependent variable are included in regression models.

relative to other potential foods and that arthropods are the most nutritionally important of all prey for promoting crayfish growth and should be selectively consumed. Crayfish in *Dreissena*-dominated habitats of the Great Lakes may adopt foraging strategies that increase their efficiency in capturing amphipods which are among the most abundant of nutritionally important prey. Although amphipods may avoid some large predators by inhabiting interstices between *Dreissena* shells, we suggest that amphipods are vulnerable to crayfish in these microhabitats. Crayfish use chelae and pereiopods to probe spaces between *Dreissena* shells for food (T.W. Stewart, personal observations), and abilities of amphipods to escape crayfish predation may be reduced if amphipods are unable to maneuver well in confined spaces between shells.

The absence of strong crayfish effects on *Dreissena* densities and shell length frequency distribution in our study contradicts results of Perry et al. (1997) who found that male *O. rusticus* (20 individuals·m^{-2}, 33–35 mm carapace length) reduced densities of all size-classes of *Dreissena* in a midwestern stream by 31% (initial *Dreissena* density = 20 225 individuals·m^{-2}, shell lengths ≤30 mm). In our experiment and that of Perry et al. (1997), crayfish species and sex were identical, cages were stocked at the same crayfish densities, and crayfish were allowed to graze for the same number of days during the same time of year (28 days during the summer months). Our crayfish were slightly smaller than those used by Perry et al. (1997) (26–32 and 33–35 mm carapace length, respectively). Thus, we did not expect crayfish in our study to eat as many large *Dreissena* as were consumed in the study by Perry et al. (1997).

Despite these potential explanations for the absence of crayfish effects on *Dreissena* in our study, we argue that crayfish effects on *Dreissena* should have been detected if *Dreissena* individuals with shell lengths ≥1 mm are important components of *O. rusticus* diets in western Lake Erie. More than 97% of *Dreissena* individuals within cages and cageless reference plots at the beginning and end of our experiment were within the size range of individuals (shell length ≤17 mm) determined by Perry et al. (1997) to be easily consumed by *O. rusticus* with carapace lengths ≤32 mm. Additionally, initial *Dreissena* densities used by Perry et al. (1997) were more than three times as great as densities within our cages and cageless plots (20 225 and 6203 individuals·m^2 horizontal benthic surface area^{-1}, respectively). We think that the absence of crayfish effects on *Dreissena* in our experiment resulted from crayfish selectively consuming amphipods to maximize energy and nutrient consumption per unit of effort.

We suggest that *Dreissena* has strong indirect positive effects on *O. rusticus* in western Lake Erie by causing increased amphipod densities, providing crayfish with an abundant, nutritionally important prey. Exploitation of *Dreissena*-generated increases in amphipod populations appears to be an important mechanism for post-*Dreissena* invasion increases in crayfish densities in the Great Lakes. Conversely, *O. rusticus* appears to have weak effects on *Dreissena* in western Lake Erie. However, crayfish diets can vary with species, sex, age, and time of year (Momot 1995). Therefore, investigations of effects of these factors are needed in conjunction with quantitative surveys of crayfish demographics for a clear understanding of crayfish effects on *Dreissena* and other potential macroinvertebrate prey in the Great Lakes.

Cage effects on crayfish and other macroinvertebrates

Because cages designed to enclose or exclude organisms in ecological experiments modify habitats, causing environments within cages to differ from natural conditions, it is important to distinguish unintended cage effects from effects of intended treatments on the dependent variables of interest (Peckarsky and Penton 1990). By including the variable "cage" in multiple regression analyses, we controlled statistically for cage effects on macroinvertebrates and also quantified the independent effects of cages on macroinvertebrates. Cages caused reductions in densities of *Dugesia* and *Ferrissia*. However, cages had negligible effects on *Dreissena* and *Gammarus*, which were by far the most important components of the macroinvertebrate community in terms of numbers and biomass.

Because crayfish forage more frequently and for longer periods of time when fish are absent (Hill and Lodge 1995), crayfish might be expected to have greater effects on macroinvertebrates within cages relative to cageless reference plots. However, we observed smallmouth bass (*Micropterus dolomieu*) bumping cages in attempts to capture enclosed crayfish, and crayfish reacted by hiding under rocks or assuming characteristic defensive positions (raising and spreading of chelae). Because of these observations, in combination with the absence of a cage effect on *Dreissena* and amphipods, we think that crayfish behavior and effects on the macroinvertebrate community were similar in both cages and cageless reference plots.

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Conclusions

The crayfish *O. rusticus* can reduce amphipod densities on *Dreissena*-dominated hard substrates in western Lake Erie. By promoting increases in amphipod densities, *Dreissena* may indirectly enhance food resources available to crayfish in rocky habitats of the Great Lakes. Because both crayfish and amphipods are prey for many species of fish (Lodge and Hill 1994; Lott et al. 1996), interactions among *Dreissena*, amphipods, and crayfish may be important pathways of energy and nutrient flow from *Dreissena* to higher trophic levels in the Great Lakes.

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On décrit dans ce document, le Bulletin canadien des sciences halieutiques et aquatiques 226 (un recueil des 15 articles), des approches de gestion de diverses pêches axées sur la diversité et la complexité des pêches maritimes canadiennes. Visitez http://www.nrc.ca/cisti/journals/books/perspe_f.html au site Web des Press scientifiques du CNRC pour consulter la table des matières.

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Numerical Methods in Atmospheric and Oceanic Modelling

The André J. Robert Memorial Volume

Charles A. Lin, René Laprise and/et Harold Ritchie (Editors/Directeurs scientifiques)

634 pages
November/Novembre 1997
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$79.95/79,95 $

This book, co-published with the Canadian Meteorological and Oceanographic Society, is a collection of papers by leading scientists in the field of numerical modelling, from the 1994 André J. Robert Memorial Symposium on Numerical Methods in Atmospheric and Oceanic Sciences. Visit http://www.nrc.ca/cisti/journals/41646/tc41646.html to view the Table of Contents.

Cet ouvrage, publié en collaboration avec la Société canadienne de météorologie and d'océanographie, est une collection d'articles, basés sur des conférences présentées par des scientifiques réputés lors du 1994 Symposium à la mémoire d'André J. Robert. Visitez http://www.nrc.ca/cisti/journals/41646/ftc41646.html pour afficher le table des matières du livre.

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Guide to the Parasites of Fishes of Canada. Part IV. Trematoda

David I. Gibson

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More than 80 keys leading to the identification of more than 220 species of trematode parasites of Canadian fishes and diagnoses of more than 80 family–group taxa and 130 genera are given. Each genus is illustrated. For each species a Canadian host list, locality data, and any relevant remarks are included. Visit http://www.nrc.ca/cisti/journals/40083/133to136.html at the NRC Research Press Web to view a short chapter of this book.

On retrouve plus de 80 clefs qui permettent d'identifier quelque 220 espèces de trématodes parasites des poissons du Canada et la description de plus de 80 taxums de famille/groupe et de 130 genres. Chaque genre y est illustré. À chaque espèce sont associées des données sur l'emplacement et des remarques particulières. Visitez http://www.nrc.ca/cisti/journals/40083/f133to6.html au site Web des Presses scientifiques du CNRC pour afficher un court chapitre du livre.

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